CARBON SOURCES AND TROPHIC CONNECTIVITY IN SEAFLOOR FOOD WEBS IN THE ALASKA ARCTIC AND SUB-ARCTIC

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A

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Abstract

Stable isotope analysis offers critical insight into organic matter pathways that sustain and link consumers in a food web. Indirect examination of organic matter sources and consumer diets using stable isotope analysis is especially valuable in the Alaska Arctic and sub-Arctic marine realm, where organisms of interest are difficult to observe given their remote habitat and elusive behavior. The research objective of this body of work was to use novel applications of stable isotope analysis to extend our understanding of organic matter sources, trophic pathways, and resource competition among benthic consumers. Microphytobenthos, a community of photosynthesizing unicellular microscopic algal cells on the seafloor sediment, has not been included in stable isotope food web models in the Alaska Arctic and sub-Arctic due to challenges associated with sample collection and analysis. I constrained the isotopic composition of this potential algal source by integrating field measurements, physiological relationships previously established by laboratory studies, and a range of algal growth rates specific to high latitude primary production. Relative to other sources of primary production in the Arctic, sub-Arctic, and lower latitude ecosystems, estimates for stable carbon isotope values of total organic carbon from microphytobenthos in the Beaufort and Chukchi seas were higher than those for Arctic riverine organic matter, but lower than ice algal sources and microphytobenthos measurements from lower latitudes. To further elucidate trophic pathways and resource partitioning among benthic invertebrate consumers, I combined compound-specific stable isotope analysis, a relatively new analytical tool, with fatty acid analysis to estimate proportional contributions of algal sources from ice, open ocean, and surface sediments to common polychaete and bivalve consumers in the Bering Sea. Benthic invertebrates were collected in 2009-2010 and represented a diverse range of feeding strategies, including the suspension/surface deposit-feeding bivalves *Macoma calcarea* and *Ennucula tenuis*, the subsurface deposit-feeding bivalve, *Nuculana radiata*, the head down deposit-feeding polychaete *Leitoscoloplos pugettensis*, and the predator/scavenger *Nephtys* spp. Differences in dominant algal sources to these invertebrate consumers corresponded, for the most part, to feeding strategy. Bivalves primarily obtained fatty acids from surface sediments, whereas *L. pugettensis* obtained fatty acids from a microbially altered phytodetrital fatty acid pool, and *Nephtys* spp. from ice algal fatty acids acquired indirectly through predation. This multi-proxy compound-specific stable isotope approach was then applied to examine dietary overlap between Pacific walruses (*Odobenus rosmarus*

divergens) and bearded seals (*Erignathus barbatus*) in 2009-2011 who feed primarily on benthic invertebrate prey. Differences in the relative proportions of fatty acids produced exclusively by benthic prey (non-methylene interrupted fatty acids) indicated that walruses and bearded seals had divergent diets. Proportional contributions of algal sources from ice, open ocean, and surface sediments to the prey consumed by walruses and bearded seals also varied. Walruses consumed prey that relied primarily on benthic and pelagic carbon sources (i.e., suspension/surface and subsurface deposit-feeding bivalves). In contrast, bearded seals consumed prey that relied on benthic and ice algal carbon sources (i.e., omnivorous and predatory benthic invertebrates). In conclusion, this research revealed that, in the recent study years, benthic food webs in the Alaska Arctic and sub-Arctic contained several trophic pathways linking consumers to distinct organic matter sources. Consequently, projected changes in algal production with future climate warming may elicit species-specific responses among benthic organisms.

Dedication

To my parents, Claire and David

For always making us go on a hike

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Acknowledgments

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General Introduction

Arctic shelf systems are among the most productive regions in the global ocean (Carmack et al. 2006). Seafloor sediments accumulate organic matter from pelagic phytoplankton, sea ice algae, and highly degraded terrestrial plants, as well as from *in situ* algal production, microbial biomass, and detrital sources (Carmack et al. 2006). These food resources support a high biomass of benthic invertebrate organisms that sustain populations of high trophic level predators, including Pacific walruses and bearded seals (Grebmeier and Cooper 1995; Bluhm and Gradinger 2008). However, the relative importance of these different organic matter sources to benthic food webs in the Arctic and sub-Arctic remains unknown.

Primary production in the Arctic and sub-Arctic consists of several algal sources that occupy distinct habitats; contributions of these sources to total annual primary production are poorly understood. These sources include phytoplankton, which grows in the water column, ice algae, which grow in the brine channels of sea ice, and microphytobenthos, which grows on the sediment surface (Horner and Schrader 1982; Arrigo et al. 2012; Boetius et al. 2013). Phytoplankton is a dominant source of algal production in the Bering and Chukchi seas. Its contribution to total annual production has increased considerably in the Chukchi Sea over recent decades due to an increase in the number of ice-free days each year (Brown and Arrigo 2012; Arrigo et al. 2012). Ice algal contributions to annual production are low to moderate (3-30 %) (McRoy and Goering 1976; Legendre et al. 1992; Tremblay et al. 2009) and vary regionally due to differences in seasonal ice cover (Gosselin el al. 1997). The proportional contribution of microphytobenthos to total annual primary production is not known for the Alaska Arctic because previous research is limited in geographic scope (Matheke and Horner 1974; Horner and Schrader 1982; Glud et al. 2009). Another algal community that has not been accounted for in total annual primary production is under-ice phytoplankton, which was recently discovered in the Chukchi Sea (Arrigo et al. 2012). In the Alaska Arctic, algal material from these sources can be subject to processes, such as lateral advection, resuspension and burial, grazing, and microbial degradation, that influence the quantity and quality of the organic matter available to benthic consumers (Grant et al. 2002; Moran et al. 2005; Morata and Renaud 2008; Cooper et al. 2009).

Given uncertainty regarding the production, modification, and availability of organic matter to benthic consumers, analytical tools that provide indirect evidence of trophic

connectivity are especially important in the Arctic marine environment. To trace organic matter sources to benthic consumers, organisms can be analyzed for their stable carbon isotopic composition (i.e., $\delta^{13}C$ values of total organic carbon-TOC), which varies minimally and predictably (0-1 ‰) from that of their diet or carbon source (Fry and Sherr 1988; Hobson and Welch 1992). Circumarctic stable carbon isotope analyses of carbon sources have revealed patterns in the isotopic composition of total organic carbon (TOC) from distinct algal sources. For example, $\delta^{13}C$ values of ice algae tend to be higher compared with those of phytoplankton because algae photosynthesizing in the brine channels of sea ice can have high growth rates yet a limited resupply of the lighter isotope of carbon (^{12}C) in the available photosynthetic substrate. In contrast, phytoplankton photosynthesizing in the water column has a steady resupply of ^{12}C so its metabolic preference for the lighter isotope is expressed and its $\delta^{13}C$ values tend to be lower than those of ice algae (e.g., Fischer 1991; Kennedy et al. 2002; Wang et al. 2014).

Although there is a fairly comprehensive dataset of $\delta^{13}C$ values to describe the range of isotopic variation in ice algal and phytoplankton sources, there are no isotopic measurements to date of microphytobenthos, microscopic algal cells that persist at the sediment-water interface and in interstitial spaces of surface sediments. Microphytobenthos from the nearshore environment in the Beaufort and Chukchi seas has been studied (Matheke and Horner 1974; Horner and Schrader 1982), but not analyzed for its stable carbon isotopic composition. Recently, microphytobenthos was successfully collected and isolated from Chukchi Sea sediments. However, the technique used to remove non-algal material influenced its stable carbon isotopic composition, making it unfeasible to obtain a reliable isotope measurement (McTigue and Dunton 2014).

In order to include microphytobenthos as a potential carbon source in stable isotope mixing models describing benthic consumer diets, I combine field measurements and empirically derived relationships describing algal physiology to estimate a range of $\delta^{13}C$ values for TOC from microphytobenthos. To constrain the stable carbon isotopic composition of microphytobenthos, I investigate the influences on modeled values of several factors: dissolved inorganic carbon (DIC) $\delta^{13}C$ values ($\delta^{13}C_{DIC}$) and DIC concentration in bottom water, algal growth rate, and morphology (algal taxon). I measure $\delta^{13}C$ values of DIC at depth (~5 m from the sediment-water interface) at field sites on the Beaufort and Chukchi shelves, a dataset that

complements the few measurements currently available from the Arctic basin and shelf (Griffith et al. 2012; Coffin et al. 2013). I then combine these field measurements with a set of empirically derived and previously published equations that express stable carbon isotope fractionation for a centric diatom, a haptophyte, and a pennate diatom species (Laws et al. 1995; Popp et al. 1998). This novel analytical approach has a few advantages. One advantage is that it mitigates the potential for contamination from meiofauna, bacteria, and benthic detritus, a common problem in lower latitude systems where microphytobenthos has been analyzed for its stable carbon isotopic composition (Oakes et al. 2005 and references therein). Another advantage is that the model allows for examination of the influence of a range of environmental and physiological variables on the stable carbon isotopic composition of microphytobenthos.

Although stable carbon isotope analysis of TOC has been used successfully to describe organic matter contributions to pelagic consumers in the Arctic (e.g., Hobson and Welch 1992; Søreide et al. 2006), recent studies reported $\delta^{13}C$ values for several benthic invertebrate taxa that fell outside the range of measured carbon sources (i.e., TOC from ice algae, phytoplankton, and surface sediments) (Iken et al. 2005; Lovvorn et al. 2005; McTigue and Dunton 2014). In Chapter 2, I use a multi-proxy compound-specific approach, which combines fatty acid (FA) analysis with stable carbon isotope analysis of algal FA markers to determine the proportional contributions of organic matter sources to benthic invertebrate consumers. This analytical approach confers a considerable advantage over stable isotope analysis of TOC because it provides multiple lines of evidence from which to link consumers to their organic matter sources (e.g., Budge et al. 2008; Wang et al. 2015). I use FA profiles (the relative proportions of all FAs from a sample), FA markers of sources (e.g., algae, bacteria), and $\delta^{13}C$ values of algal marker FAs to ascertain whether benthic invertebrate organisms with unique feeding strategies obtained organic matter from distinct algal sources in the Bering Sea. I select five focal organisms, all of which are common in the Bering Sea benthos and collectively represent a diverse set of feeding strategies. These include suspension/surface deposit-feeders (the bivalves *Macoma calcarea* and *Ennucula tenuis*), a subsurface deposit-feeder (the bivalve *Nuculana radiata*), a head-down deposit-feeder (the polychaete *Leitoscoloplos pugettensis*), and a predator/scavenger (the polychaete *Nephtys* spp.).

Benthic invertebrate taxa are a critical component of benthic food webs because they transfer energy and nutrients from primary producers to apex predators. Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*) consume a broad variety of benthic prey, including bivalves, polychaetes, gastropods, shrimp and crabs (Lowry et al. 1980; Fay 1982; Sheffield and Grebmeier 2009). In 1980, Lowry and colleagues attributed evidence of stress in the Pacific walrus population to competition with bearded seals over benthic food resources, specifically clams (Lowry et al. 1980). The potential for population stress due to resource competition could be even greater in recent years given the steady decline in Arctic sea ice cover observed since 1980 (Stroeve et al. 2007; Comiso et al. 2008).

Changes in the ice environment could limit the geographic extent of foraging by walruses and potentially increase competition for dietary resources between walruses and bearded seals. Extensive sea ice decline as well as a temporal shift in seasonal sea ice retreat (Grebmeier et al. 2006) have important implications for these ice-associated marine mammals, which rely on sea ice habitat for various life history patterns including molting, mating, and giving birth (Kovacs et al. 2011). Sea ice is especially important for Pacific walruses, which use sea ice as a diving platform from which to access benthic prey during foraging bouts (Ray et al. 2006). In recent years, summer sea ice has receded north of the Chukchi shelf over the Arctic Basin, which is too deep for walrus foraging (Jay and Fischbach 2008). As a result, walruses have increased their use of terrestrial haul out locations on the northwest coast of Alaska to rest between foraging bouts. Increased reliance on terrestrial haul outs could change the energy budgets of walruses or, alternatively, the spatial extent of their foraging. While bearded seals do not rely on the pack ice for foraging (Cameron et al. 2010), a change in foraging patterns by walruses could affect competition for prey resources and place additional pressure on species already affected by ice loss (Bluhm and Gradinger 2008; Jay and Fischbach 2008; Jay et al. 2012, 2014).

In Chapter 3, I use the same multi-proxy compound-specific approach as described in Chapter 2 to examine dietary overlap between Pacific walruses and bearded seals. Using FA profiles, FA markers of sources (e.g., algae, bacteria, select benthic invertebrates), I examine the extent to which these predators partitioned resources in the benthos. I then use $\delta^{13}C$ values of algal marker FAs to estimate the proportional contributions of distinct algal sources to the prey walruses and bearded seals consumed. To interpret dietary differences, I compare the $\delta^{13}C$ values

of algal FAs from walruses and bearded seals to those of a diverse group of benthic invertebrate prey collected from the Bering and Chukchi seas.

In summary, this dissertation consists of three studies that use an innovative application of stable isotope analysis to extend our understanding of organic matter sources, trophic pathways, and resource use among consumers in seafloor food webs. Studies were carried out in recent years (2009-2012) in the Alaska Pacific Arctic and sub-Arctic, including the Beaufort (Chapter 1), Chukchi (Chapters 1-3), and Bering (Chapters 2, 3) seas. The broad goal of this research is to investigate the transfer of energy and nutrients through benthic food webs in the Arctic and sub-Arctic.

Chapter 1 Estimating stable carbon isotope values of microphytobenthos in the Arctic for application to food web studies [1]

1.1 Abstract

Most studies on Arctic food webs have neglected microphytobenthos as a potential food source because we currently lack robust measurements of $\delta^{13}C$ values for microphytobenthos from this environment. As a result, the role of microphytobenthos in high latitude marine food webs is not well understood. We combined field measurements of the concentration of aqueous carbon dioxide and the stable carbon isotopic composition of dissolved inorganic carbon ($\delta^{13}C_{DIC}$) from bottom water in the Beaufort and Chukchi seas with a set of stable carbon isotopic fractionation factors reflecting differences in algal taxonomy and physiology to estimate the stable carbon isotope composition of microphytobenthos-derived total organic carbon (TOC) ($\delta^{13}C_p$). The $\delta^{13}C_p$ for *Phaeodactylum tricornutum*, a pennate diatom likely to be a dominant microphytobenthos taxon, was estimated to be -23.9 ± 0.4 ‰ as compared to a centric diatom (*Porosira glacialis*, $\delta^{13}C_p$ = -20.0 ± 1.6 ‰) and a marine haptophyte (*Emiliana huxleyi*, $\delta^{13}C_p$ = -22.7 ± 0.5 ‰) at a growth rate (μ) of 0.1 divisions per day (d^{-1}). $\delta^{13}C_p$ values increased by ~2.5 ‰ when μ increased from 0.1 to a maximum growth rate of 1.4 d^{-1}. We compared our estimates of $\delta^{13}C_p$ values for microphytobenthos with published measurements for other carbon sources in the Arctic and sub-Arctic. We found that microphytobenthos values overlapped with pelagic sources, yet differed from riverine and ice-derived carbon sources. These model results provide valuable insight into the range of possible isotopic values for microphytobenthos from this region, but we remain cautious in regard to the conclusiveness of these findings given the paucity of field measurements currently available for model validation.

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[1] Oxtoby, L.E., Mathis, J.T., Juranek, L.W., Wooller, M.J. (2016) Estimating stable carbon isotope values of microphytobenthos in Arctic food webs for application to food web studies. Polar Biology 39:473-483 doi: 10.1007/s00300-015-1800-2

1.2 Introduction

Projected impacts of climate change and industrial development on the marine environment necessitate an improved understanding of energy flow and food web structure in the Arctic (Carmack et al. 2006). Stable carbon isotope analyses of total organic carbon (TOC) from organisms can provide an effective tool to determine contributions from different primary production sources to Arctic food webs (Hobson et al. 2002; Budge et al. 2008; Dunton et al. 2012). Typically assessed primary producer sources in Arctic food web studies are pelagic, riverine, and sympagic organic matter. These sources differ in their $\delta^{13}C$ values due to variation in the composition and availability of the carbon source used in photosynthesis. Pelagic phytoplankton obtain dissolved inorganic carbon (DIC) from surface ocean waters where the global mean stable carbon isotope composition ($\delta^{13}C_{DIC} = 1.5 \pm 0.8$ ‰) (Gruber et al. 1999) is enriched in ^{13}C relative to terrestrial sources such as atmospheric CO_2 ($\delta^{13}C_{atm} = -7.9$ ‰) (Farquhar et al. 1989). Riverine organic matter $\delta^{13}C$ values are low compared to marine sources because riverine organic matter consists largely of highly degraded terrestrial C3 plants, including tundra taiga and angiosperms, that fix atmospheric CO_2 (Naidu et al. 1993; Goñi et al. 2000, 2005). Sympagic organic matter can have a unique stable carbon isotope composition relative to pelagic and riverine sources due to limited exchange of DIC in the brine channel matrix (e.g., Fischer 1991; Kennedy et al. 2002; Wang et al. 2014). At high levels of photosynthesis in a closed or semi-closed system, restricted exchange results in decreased expression of isotopic fractionation (Hobson et al. 1995; McMahon et al. 2006; Søreide et al. 2013).

Microphytobenthos is often not included as a potential source of primary production to Arctic food webs despite its prevalence on shallow shelves in the Arctic (Matheke and Horner 1974; Horner and Schrader 1982; Glud et al. 2009). Microphytobenthos is a distinct algal community dominated by pennate diatoms in the Arctic that develops exclusively on the sediment surface (Glud et al. 2009; Wulff et al. 2009 and references therein). Due to challenges associated with sample collection in the Arctic, including limited access to shallow stations on oceanographic field campaigns, the separation of microphytobenthos-derived organic matter from sediment samples and direct measurements of its isotopic composition are rare.

At lower latitudes, the isotopic composition of microphytobenthos is well characterized based on actual measurements (France 1995 and references therein). Techniques such as centrifugation in colloidal silica (Blanchard 1990), sediment scrapes of microphytobenthos colonies, collection of gut contents from known consumers of microphytobenthos, and additional methods reviewed by Oakes et al. (2005) have been used in temperate, tropical, and subtropical systems to isolate microphytobenthos for bulk and compound-specific stable isotope analysis (e.g., Oakes et al. 2005 and references therein; Evrard et al. 2010; Oakes et al. 2010a). However, robust measurements of the isotopic composition of microphytobenthos are difficult to perform due to the potential for contamination from additional organic matter sources such as microbial biomass, meiofauna, and detritus. To avoid the introduction of impurities associated with extant sampling techniques, innovative compound-specific predictive modeling approaches have been employed in order to constrain estimates for microphytobenthos $\delta^{13}C$ values (Evrard et al. 2010; Oakes et al. 2010b; Evrard et al. 2012).

In the Arctic, predictive models are also especially useful because little is known about the spatial distribution of microphytobenthos, making sample collection difficult and, most always, opportunistic. To our knowledge, there are no published microphytobenthos stable isotope values from the Arctic Ocean (our study region). In a recent study, McTigue and Dunton (2014) were able to isolate microphytobenthos from Chukchi Sea sediments using a method developed by Blanchard (1990). However, they were unable to produce a reliable isotope measurement of the sample to include as an additional isotopic end member in their study.

Ideally, microphytobenthos sample analysis and predictive modeling approaches would be used in concert to produce a confident estimate of microphytobenthos isotopic composition. Combined results from actual measurements, predictive models for microphytobenthos, and isotopic labeling in lower latitude environments have provided insight into broad ecological questions regarding organic matter pathways and contributions of carbon sources to benthic consumers (e.g., Middelburg et al. 2000; Evrard et al. 2010; Van den Meersche et al. 2011). Microphytobenthos stable isotope research from studies conducted at lower latitudes provides direction for future research efforts in the Arctic and sub-Arctic.

We present an approach that estimates the stable carbon isotopic composition of microphytobenthos (TOC) from coastal regions of the Beaufort and Chukchi seas for future

consideration in Arctic food web studies. The central objective of our study was to identify the bounds for estimates of $\delta^{13}C$ values of TOC derived from microphytobenthos, given variation in DIC composition and availability and algal taxonomy and physiology. First, we measured the concentrations and stable carbon isotopic compositions (expressed here as $\delta^{13}C$ values) of DIC in bottom water samples from the Beaufort shelf. We then used empirically-derived quantitative relationships between the $\delta^{13}C$ values of DIC, the aqueous concentration of CO_2 ($[CO_2]aq$) in seawater, and a range of previously reported photosynthetic fractionation factors (ε_p) (Laws et al. 1995; Popp et al. 1998) that account for differences in algal taxonomy, morphology and growth rate (μ) to constrain the $\delta^{13}C$ values of microphytobenthos TOC ($\delta^{13}C_p$) in this region. We compared these estimates to $\delta^{13}C$ values of previously measured carbon sources (i.e., pelagic, riverine, sympagic) in the Arctic and to $\delta^{13}C_p$ from lower latitudes.

1.3 Materials and methods

1.3.1 Sample collection and preparation

Seawater samples (n = 18) were collected from ~5 m above the sediment-water interface along four transects in the Beaufort and Chukchi seas in October 2012 during a research cruise on the USCGC Healy (HLY1203) (additional information available in Appendix 1.1). Transects were located at the mouths of Barrow Canyon and the Mackenzie River, to the east of Point Barrow, and across Amundsen Strait (Fig. 1.1). Water depth ranged from 28 to 346 m. At each station sampled (n = 18), a CTD rosette (Seabird 911plus system using dual temperature, conductivity, and oxygen sensors) was deployed to record conductivity, temperature, pressure, transmittance, and fluorescence measurements on downcasts (data are available in Appendix 1.1) and to collect water samples in Niskin bottles linked to the CTD rosette. Samples for DIC isotope analyses were transferred from the Niskin bottles to 300-mL borosilicate bottles pre-cleaned with a 10 % solution of HCl without headspace or introduction of bubbles. Samples were immediately poisoned with 100 µL of mercuric chloride ($HgCl_2$) to suspend biological activity. Samples were then wrapped in Teflon tape, closed with a screw-on cap, and stored in the dark at room temperature (25 °C). Seawater samples were also taken from Niskin bottles for shipboard measurements of DIC concentration (poisoned as previously described), total alkalinity (TA), and nutrient analyses. Nutrient samples were stored frozen at -20 °C in plastic vials for subsequent analysis of nitrate, nitrite, phosphate, silicic acid, and ammonium.

1.3.2 Sample analysis

Nutrient samples were analyzed at the University of Alaska Fairbanks (UAF) using an Alpkem Flow Solution IV Autoanalyzer (OI Analytical, College Station, TX) (Whitledge et al. 1981). Analytical precision for triplicate nutrient measurements was between 0.03 and 0.05 µmol kg^{-1}. Commercially available certified standards (Ocean Scientific International and Wako Chemical), used for instrumental calibration, were included in the sample run as quality control. Shipboard measurements of DIC concentration (µmol kg^{-1}) were performed using a gas extraction/coulometric detection system that consisted of a VINDTA 3C (Versatile Instrument for the Detection of Total Alkalinity) (Marianda Co., Kiel, Germany) interfaced with a CO_2 coulometer (coulometer 5011, UIC Inc., USA). TA (µmol kg^{-1}) was measured by potentiometric titration with HCl (see Bates 2001 for details) using the same VINDTA system. Analytical precision was tracked using repeated measurements of Certified Reference Materials (CRMs, provided by A.G. Dickson, Scripps Institution of Oceanography) and was within 0.02 % (±0.4 µmol kg^{-1}).

Stable carbon isotope analyses of DIC samples were conducted at the Stable Isotope Laboratory at Oregon State University (OSU) following the methods of Torres et al. (2005). Seawater was transferred to Labco exetainer vials (7 mL), closed with rubber septa, and cooled to 13 °C in a water bath for 15 minutes. Samples were flushed with He (Matheson UHP grade) for 5 minutes, then acidified with ~0.1 mL of 85 % orthophosphoric acid (EMD Chemicals HPLC grade). Samples were allowed to equilibrate for 10 hours before stable carbon isotope analysis. DIC samples were analyzed using a Finnigan GasBench II interfaced with a Delta V Plus (Thermo Fisher Scientific, Bremen, Germany) continuous-flow isotope ratio mass spectrometer (CF-IRMS). Instrumental calibration was based on calcium carbonate (solid) international laboratory standards (NBS19 and NBS20). An internal laboratory standard (3 mM sodium bicarbonate in solution) that could be analyzed in the same way as the water samples was used for secondary calibration (Torres et al. 2005). Analytical precision was ± 0.04 ‰, expressed as 1 standard deviation (s.d.) calculated from replicate (n = 10) analyses of aqueous 3 mM sodium bicarbonate (internal laboratory standard) performed throughout the sample run. Sample precision (n = 3, station 48, expressed as 1 s.d.) was ± 0.01 ‰. Sample reproducibility, calculated from replicate (n = 11) sample analyses was ± 0.06 ‰ (expressed as 1 s.d.). Stable

carbon isotope compositions of DIC are expressed using conventional delta (δ) notation in parts per thousand (‰) based on the following equation (Eq. 1.1):

$$(1.1) \quad \delta X=[(R_{sample}/R_{standard})-1]*1000$$

where $\delta X=\delta^{13}C$, R is the ratio of $^{13}C/^{12}C$ in seawater, and $R_{standard}$ is that of the standard reference material Vienna Pee Dee Belemnite (VPDB).

1.3.3 Calculations

CO_2 concentration ($[CO_2]aq$, μmol kg^{-1}) was calculated using CO2SYS version 1.05. DIC, TA, temperature, salinity, phosphate, and silicate data were input using the thermodynamic model, dissociation constants, and solubility equations following Lewis and Wallace (1995).

Fractionation factors for C_3 photosynthesis (ε_p) were modeled using a suite of equations that describe the relationship between $[CO_2]aq$, algal growth rate μ (d^{-1}), and ε_p (Laws et al. 1995; Popp et al. 1998). Laws et al. (1995) expressed ε_p in terms of μ and $[CO_2]aq$, ($r^2 = 0.97$, $n = 5$) as follows (Eq. 1.2):

$$(1.2) \quad \mu/[CO_2]aq = -0.015* \varepsilon_p + 0.371$$

In a subsequent study, Popp et al. (1998) examined the influence of cell geometry on ε_p for a diverse group of algal taxa, all of which occur in the sub-Arctic and Arctic marine environments (Krebs 1983; Medlin et al. 1996; von Quillfeldt et al. 2003; Smyth et al. 2004): *Porosira glacialis* (centric diatom), *Emiliana huxleyi* (haptophyte), and *Phaeodactylum tricornutum* (pennate diatom). At $\mu > 0$, differences in algal morphology influence carbon ($[CO_2]aq$) supply and demand, resulting in species-specific ε_p (Popp et al. 1998). Empirically-derived regression relationships have been determined to describe the term ε_p for a centric diatom (*P. glacialis*, $\varepsilon_p^a = 25.5 - 1118.2\ \mu/[CO_2]aq$, $\mu = 0.3\ d^{-1}$, $r^2 = 0.75$, $n = 7$), a marine haptophyte (*E. huxleyi*, $\varepsilon_p^b = 24.6 - 137.9\ \mu/[CO_2]aq$, $\mu = 0.6\ d^{-1}$, $r^2 = 0.87$, $n = 9$), and a pennate diatom (*P. tricornutum*, $\varepsilon_p^c = 25.5 - 52.6\ \mu/[CO_2]aq$, $\mu = 1.4\ d^{-1}$, $r^2 = 0.78$, $n = 8$) (Popp et al. 1998). Superscripts a-c for species-specific ε_p correspond to $\delta^{13}C_p$ superscripts in Table 1.1.

We also calculated ε_p for the pennate diatom, *P. tricornutum*, exposed to the range of $[CO_2]aq$ and $\delta^{13}C_{DIC}$ observed at our field sites at three growth rates (μ = 0.1 d^{-1}, μ = 0.4 d^{-1}, μ = 1.4 d^{-1}). We selected the pennate diatom as representative of microphytobenthos due to its relative dominance in polar microphytobenthos community assemblages. We investigated changes in ε_p over a range of typical growth rates given low levels of irradiance and cold temperatures in polar environments (Longhi et al. 2003; Karsten et al. 2006). Although growth rates for polar benthic diatoms are typically μ = 0.3-0.5 d^{-1}, growth rates as high as μ = 1.24 d^{-1} have been observed (Longhi et al. 2003; Karsten et al. 2006). We selected μ = 1.4 d^{-1} as the upper limit for algal growth rate following Laws et al. (1995) because μ rarely exceeds two doublings per day in the natural environment (Laws et al. 1987). Additionally, the maximum algal growth rate observed in the Arctic during a highly productive under-ice phytoplankton bloom was 1.44 d^{-1} in the Chukchi Sea (Arrigo et al. 2012). This value is likely the absolute maximum growth rate (and may be an overestimate of ambient rates of growth) given strong light attenuation at depth and high sediment loading on the Beaufort Shelf.

Fractionation factors (ε_p) can then be used to determine the stable carbon isotope composition of bulk algal biomass ($\delta^{13}C_p$), following the theoretical relationship between the stable isotopic compositions of the carbon source ($\delta^{13}C_{DIC}$) and product ($\delta^{13}C_p$) for photosynthesis (Eq. 1.3):

$$(1.3) \quad \varepsilon_p = 1000*(\delta^{13}C_{DIC} - \delta^{13}C_p)/(1000+ \delta^{13}C_p)$$

Significant differences among $\delta^{13}C_p$ values estimated for *P. tricornutum* at low (μ = 0.1 d^{-1}), intermediate (μ = 0.4 d^{-1}), and maximum (μ = 1.4 d^{-1}) growth rates were identified using a one-way analysis of variance (ANOVA) with growth rate as the factor in conjunction with Tukey post-hoc test which corrects for family-wise error rate.

Unfortunately, there are few concurrent measurements of more than two of the parameters ($\delta^{13}C_{DIC}$, $\delta^{13}C_p$, $[CO_2]aq$, and μ) needed to validate our model, even at lower latitudes. To provide support for our model results, we first calculated a "fractionation factor" derived from the difference between $\delta^{13}C_{DIC}$ and $\delta^{13}C_p$ values measured in a subtropical subtidal shallow environment (Oakes et al. 2012). As such, the "fractionation factor" (ε_p = 18.2 ‰)

reflects growth conditions specific to microphytobenthos such as limited DIC exchange through the sediment-water interface and competition for DIC within microphytobenthos biofilms (Oakes et al. 2012). We then applied this "fractionation factor" in concert with porewater $\delta^{13}C_{DIC}$ data from our study region (Coffin et al. 2013) to our model to estimate a $\delta^{13}C_p$ value and compared it to those we calculated in this study (Table 1.1). We selected porewater $\delta^{13}C_{DIC}$ values ($\delta^{13}C_{DIC}$ = -6 ± 4 ‰, mean ± s.d., n = 11) as the carbon source for microphytobenthos ($\delta^{13}C_p$) in this validation exercise because it is a more probable source of inorganic carbon to microphytobenthos than bottom water. Porewater $\delta^{13}C_{DIC}$ values were reported in a study conducted in close proximity to our study sites in Beaufort Sea during the same time of year (Coffin et al. 2013).

1.4 Results

$[CO_2]aq$ ranged from 17 to 72 μmol kg^{-1} and $\delta^{13}C_{DIC}$ values varied from -0.1 to 1.4 ‰ (0.8 ± 0.4, mean ± 1 s.d.) for samples of bottom water at our sampling locations in the Beaufort and Chukchi seas (Table 1.1; Appendix 1.1). The lowest $[CO_2]aq$ were observed near the mouth of the Mackenzie River and corresponded to the highest $\delta^{13}C_{DIC}$ values (Stations 68-71, Fig. 1.1, Appendix 1.1). The highest $[CO_2]aq$ was observed in Barrow Canyon and corresponded to the lowest $\delta^{13}C_{DIC}$ value (Station 14, Fig. 1.1, Appendix 1.1). For sites at depths shallower than 200 m, there was an inverse correlation between $\delta^{13}C_{DIC}$ and depth (r = -0.80, n = 14). Samples from Barrow Canyon did not follow this depth gradient (Stations 14, 17, Appendix 1.1).

Based on our field measurements of $[CO_2]aq$ and $\delta^{13}C_{DIC}$ and a low algal growth rate (μ = 0.1 d^{-1}), modeled $\delta^{13}C_p$ values were highest for the centric diatom (*P. glacialis*, $\delta^{13}C_p{}^a$ = -20.0 ± 1.6 ‰), relative to those for the haptophyte (*E. huxleyi*, $\delta^{13}C_p{}^b$ = -22.7 ± 0.5 ‰), and the pennate diatom species (*P. tricornutum*, $\delta^{13}C_p{}^c$ = -23.9 ± 0.4 ‰) (Table 1.1; Fig. 1.2). For the pennate diatom (*P. tricornutum*), increasing the growth rate from low (μ = 0.1 d^{-1}) and intermediate (μ = 0.4 d^{-1}) levels to a maximum growth rate (μ = 1.4 d^{-1}) resulted in significantly higher $\delta^{13}C_p$ values (one-way ANOVA, F = 47.49, p < 0.0001) (Table 1.1) with an increase of 2.5 ‰ over the growth range. Mean $\delta^{13}C_p$ at μ = 0.4 d^{-1} was not significantly different from those calculated at μ = 0.1 d^{-1} (p = 0.09, Tukey's post-hoc test).

The mean $\delta^{13}C_p$ value for the pennate diatom ($\delta^{13}C_p^c$ = -23.3 ± 0.6 ‰, μ = 0.4 d^{-1}), an algal taxon likely to be a dominant constituent of microphytobenthos, was more enriched in ^{13}C relative to previously reported values of riverine and estuarine TOC, including benthic-POM (b-POM) from river sediments and pelagic-POM from Arctic rivers and lagoons feeding into the Beaufort Sea (riverine p-POM) (Fig. 1.2). It was depleted in ^{13}C relative to ice algae and from sea ice particulate organic matter (i-POM). Although the mean $\delta^{13}C_p$ value for the pennate diatom was enriched in ^{13}C relative to marine p-POM from regions of low productivity such as the Canada Basin, it fell between reported ranges for most values for marine p-POM from the Beaufort and Chukchi seas and from neighboring regions in the Arctic (Fig. 1.2). Relative to reported values from studies conducted at lower latitude ($\delta^{13}C$ = -17 ± 4 ‰) (France 1995 and references therein), model estimates were depleted in ^{13}C.

When previously published data were applied to the model (see "Materials and methods" section for additional information on the model validation exercise), our model predicted a $\delta^{13}C_p$ value ($\delta^{13}C_p$ = -23.8 ‰) that is consistent with those we report for the pennate diatom taxon ($\delta^{13}C_p^c$ = -23.9 ‰) (Table 1.1).

1.5 Discussion

The primary aim of this study was to estimate the stable carbon isotopic composition of the microphytobenthic community in Arctic waters in order to assess their potential incorporation in stable isotope food web studies. Microphytobenthos, a potential source of primary production to benthic food webs (Glud et al. 2009; Oakes et al. 2010a; Alderson et al. 2013), has rarely been considered in stable isotopic food web studies in the Arctic because it has not been described isotopically.

Stable isotopic analyses of microphytobenthos and DIC from bottom water and porewater from high latitude environments are necessary to determine whether our predictive model estimates are accurate. These isotopic measurements could serve as a validation to evaluate model behavior and adjust regression relationships used to model $\delta^{13}C_p$ values. Sample collection presents many challenges in the Arctic, given the nature of field sampling and the patchy distribution of microphytobenthos. Most oceanographic campaigns, including the one for this study, are carried out using research vessels in offshore waters. Marine coastal areas where microphytobenthos does occur are usually difficult to sample because of their shallow depth

(e.g., Matheke and Horner 1974; Dunton et al. 2012). We were unable to collect microphytobenthos samples in concert with our bottom water DIC samples at study sites in the Beaufort and Chukchi seas due to these logistical constraints.

In the absence of comparative data from the Arctic or from microphytobenthos culture studies, our predictive modeling approach relies on several assumptions that cannot be fully corroborated at present. Empirical relationships from our model were developed from data for pelagic phytoplankton (suspended cells) (Laws et al. 1995; Popp et al. 1998), so we remain cautious in regard to conclusions from our findings. Although it is widely accepted that $\delta^{13}C$ values for benthic algae from marine coastal areas are, on average, more enriched than pelagic algae (France 1995), differences in $\delta^{13}C$ values of local DIC, availability of an inorganic carbon source ($[CO_2]aq$), algal growth rate, and microphytobenthos composition produce microphytobenthos values that deviate from this trend, as is evidenced by the range observed in more recent studies (Oakes et al. 2010a, b; Evrard et al. 2012). A potential difference between phytoplankton and microphytobenthos that could influence the fractionation factor (ε_p) and result in different $\delta^{13}C_p$ for phytoplankton and microphytobenthos is variation in growth rate. There is evidence to suggest, however, that phytoplankton growth rates are the same as, if not higher than, those for microphytobenthos growing in polar regions, where cold temperatures, low nutrient availability, and light limitations depress algal growth (Kirst and Wiencke 1995). The maximum growth rate used by Laws et al. (1995) and by this study ($\mu = 1.4\ d^{-1}$) was substantially higher than the maximum growth rate observed for polar microphytobenthos ($\mu = 1.24\ d^{-1}$) (Longhi et al. 2003; Karsten et al. 2006).

Stable isotopic variation between pelagic and benthic microalgae has also been attributed to differences in DIC availability and composition (France 1995; Hecky and Hesslein 1995). We might expect microphytobenthos to be isotopically distinct from pelagic sources given distinct benthic conditions such as DIC limitation in the benthic boundary layer at the seafloor (France 1995; Hecky and Hesslein 1995). Our use of $\delta^{13}C_{DIC}$ values from bottom water to constrain estimates for $\delta^{13}C_p$ could bias modeled values if porewater and bottom water DIC pools are isotopically distinct. However, recent evidence from our study region along the Alaska shelf of the Beaufort Sea suggests that porewater DIC is not isotopically distinct from our bottom water measurements (Table 1.1) (Coffin et al. 2013). Porewater $\delta^{13}C_{DIC}$ measurements were made

during the same time of year as our bottom water sample collection for isotopic analysis (Coffin et al. 2013). In all locations, porewater $\delta^{13}C_{DIC}$ values near the sediment water interface, where microphytobenthos would be growing, were very similar to those of typical seawater values (Coffin et al. 2013). Moreover, the range of $\delta^{13}C_{DIC}$ values reported for porewater from varying sediment depths ($\delta^{13}C_{DIC}$ = -6 ± 4 ‰, mean ± s.d., n = 11) was the same as, or more depleted than, our DIC values from bottom water. This gives us confidence that our measured $\delta^{13}C_{DIC}$ values are appropriate to constrain estimates for microphytobenthos biomass. Additional porewater and bottom water sampling in the Arctic would bolster our estimates and elucidate a poorly studied compartment of the benthic carbon cycle.

Given the necessary assumptions for our modeling approach, our experimental results are a first step towards assessing the potential incorporation of microphytobenthos into marine Arctic food web studies with the hope that additional studies will refine this approach. Although we report some variability in $\delta^{13}C_{DIC}$ values and $[CO_2]aq$ across our study region, the ranges have little influence (~1.6 ‰) on modeled $\delta^{13}C_p$ values for the dominant algal constituent of microphytobenthos (pennate diatoms) (Horner and Schrader 1982) (Table 1.1).

Mean $\delta^{13}C$ values for microphytobenthos from lower latitude marine coastal sites (France 1995) were, on average, enriched in ^{13}C relative to those we report here. In the subtropics, there is considerable variation in microphytobenthos $\delta^{13}C_p$ values from photic sediments, from highly enriched values ($\delta^{13}C_p$ = -14.3 ± 0.6 ‰) (Oakes et al. 2014) to values more depleted than those we determine here ($\delta^{13}C_p$ = -25.5 ± 1.0 ‰) (Oakes et al. 2010a). In some cases, it is not possible to resolve benthic (microphytobenthos) production in lower latitude systems due to the presence of algal taxa in the microphytobenthos assemblage (e.g., cyanobacteria and green algae), which resemble other sources (e.g., pelagic suspended particulate matter) (Evrard et al. 2012). Whereas isotopic measurements of microphytobenthos from lower latitude ecosystems integrate $\delta^{13}C_p$ values from multiple algal taxa (and from potential contaminants), our model describes variation in $\delta^{13}C_p$ values for individual algal taxa.

Low variability in our $\delta^{13}C_p$ estimates for individual microphytobenthos taxa also reflects the narrow range of $\delta^{13}C_{DIC}$ values we observed from bottom water from this region. $\delta^{13}C_{DIC}$ values have been described for surface waters in the world ocean as part of the Geochemical

Ocean Sections (GEOSECS) program (Gruber et al. 1999) and, more recently, at varying depths in the Arctic Ocean (Griffith et al. 2012). Global measurements of $\delta^{13}C_{DIC}$ values, which are very consistent across regions ($\delta^{13}C_{DIC} = 1.5 \pm 0.8$ ‰), were slightly enriched compared to those observed in our study ($\delta^{13}C_{DIC} = 0.8 \pm 0.4$ ‰). Griffith et al. (2012) reported a range of $\delta^{13}C_{DIC}$ values (0.13 to 1.63 ‰) from off-shelf sites in the Canada Basin that are in agreement with those we observed. In addition to expanding spatial coverage for $\delta^{13}C_{DIC}$ measurements at depth in the Arctic, our measurements narrow the sampling gap between surface waters and porewater (Coffin et al. 2013).

DIC measurements from this study revealed statistically significant depth-dependent gradients in $[CO_2]aq$ and $\delta^{13}C_{DIC}$ values wherein deeper sites contained higher $[CO_2]aq$ and depleted $\delta^{13}C_{DIC}$ values relative to shallower sites (Table 1.1; Fig. 1.1). An exception to this pattern was the Barrow Canyon transect, which is hydrographically and biologically distinct from the other Beaufort shelf sites (Pickart et al. 2009). Variation in $\delta^{13}C_{DIC}$ values can be explained by processes involving preferential uptake of the light stable isotope of carbon (^{12}C) (e.g., biological production) and those that release it into the DIC pool (e.g., carbon remineralization) (Holmden et al. 1998; Gruber et al. 1999) and by contributions from isotopically distinct sources such as riverine DIC (Macdonald et al. 2004). In the marine environment, biological production and carbon remineralization occur largely in surface waters and at the seafloor, respectively, creating a depth-dependent gradient in $\delta^{13}C_{DIC}$ values (Emerson and Hedges 2008).

$\delta^{13}C_{DIC}$ values can also be a useful indicator of DIC source given observed differences in $\delta^{13}C_{DIC}$ values from riverine and marine sources (Patterson and Walter 1994). To this end, one might have expected the Mackenzie River delta transect, where riverine organic material enters the Arctic Ocean (Macdonald et al. 2004) to have the lowest $\delta^{13}C_{DIC}$ values. Contrary to this expectation, $\delta^{13}C_{DIC}$ values at the Mackenzie River delta were most isotopically enriched in ^{13}C relative to other sampling locations. These relatively high $\delta^{13}C_{DIC}$ values corresponded to the lowest $[CO_2]aq$, possibly indicating that elevated benthic primary production resulted in subsequent depletion of $[CO_2]aq$ and drawdown of isotopically light DIC.

Elevated benthic primary production (growth rate) can also influence $\delta^{13}C$ values of microphytobenthos and is often mediated by environmental conditions, such as light, temperature, and nutrient availability (Fry and Wainright 1991; Kirst and Wiencke 1995; Korb et al. 1996; Pancost et al. 1997). Based on light limitation at depth and the maximum depth for microphytobenthos growth previously reported (Cahoon et al. 1990; Cahoon 1999; McGee et al. 2008), we would expect the contribution of microphytobenthos to be greatest at shallow sites (i.e., stations 23, 79, 71) and at coastal locations that we were unable to access in the field. Palmer et al. (2013) measured 0.1 % light depth (euphotic depth) to be 37 ± 18 m in the Beaufort and Chukchi seas during the months of June and July under open water and under sea ice. This gives us confidence that considerable microphytobenthos growth could occur at stations < 100 m (Appendix 1.1, $n = 11$) and across much of the Chukchi and Beaufort seas due to their wide, shallow shelves. However, we do not expect microphytobenthos growth at stations > 100 m depth (Appendix 1.1, $n = 6$) where low light availability would limit photosynthesis.

We determined that within a selected growth range, $\delta^{13}C_p$ values for the dominant algal constituent of microphytobenthos (pennate diatoms) increased on the order of approximately 2.5 ‰. This indicates that isotopic values for microphytobenthos may vary seasonally but within a relatively small range (Fig. 1.2). Seasonal variability in $\delta^{13}C_p$ values may be pronounced, however, if algal community succession occurs in the benthos as in the pelagic realm during the course of the growing season (Moran et al. 2012) because individual taxa had distinct modeled values (Table 1.1; Fig. 1.2). Differences in the isotope values of algal taxa may be the result of varying expression of carbon concentrating mechanisms (CCM), which have been observed in marine algae (mainly diatoms) (Giordano et al. 2005; Haimovich-Dayan et al. 2013). C3 photosynthesis, as modeled here, is considered the predominant biochemical pathway for production in marine algae (Haimovich-Dayan et al. 2013 and references therein). However, biophysical and biochemical CCMs could result in variable fractionation factors and isotopically enriched algal organic matter relative to that of its inorganic carbon source.

Additionally, differences in fractionation in distinct algal taxa may result from variation in RubisCO, the carbon dioxide fixation enzyme. Algal taxa use at least four known forms of RubisCO (Ishida and Green 2002). Boller et al. (2011) measured isotopic discrimination at the enzyme level of a form of RubisCO from *E. huxleyi*. This form of RubisCO is also the dominant

form in diatoms, rhodophytes, and certain dinoflagellate species (Ishida and Green 2002). It was characterized by low isotopic discrimination (ε = 11.1 ‰) relative to previously published values for additional enzymatic forms (ε = 18-29 ‰) (Boller et al. 2011 and references therein) and relative to whole cell fractionation factors we report based on our field measurements (ε = 21.2 to 25.3 ‰, Table 1.1).

In summary, we provide model estimates of the $\delta^{13}C$ values of TOC originating from microphytobenthos in the Arctic. We also report a narrow distribution of $\delta^{13}C$ values of DIC and provide measurements of $[CO_2]aq$ from bottom water across the Beaufort and Chukchi seas during the onset of winter. Based on published $\delta^{13}C$ values of TOC from other sources of primary production in the Arctic and sub-Arctic, we suggest that $\delta^{13}C$ values of microphytobenthos may be distinct from those of riverine and sympagic origins, and from marine p-POM under conditions of low productivity. However, the stable carbon isotope composition of microphytobenthos was indistinguishable from that of marine p-POM under conditions of high productivity. Compared to previously reported microphytobenthos $\delta^{13}C$ values from studies outside of the Arctic, microphytobenthos values predicted by our model were depleted in ^{13}C. Further sample collection and analysis of microphytobenthos in the Arctic and sub-Arctic in combination with data from culture studies is of critical importance to investigate these differences and to improve this predictive model.

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conducted fieldwork and sample analysis. LWJ and JTM provided editorial advice. MJW provided ideas for data analysis and presentation and greatly assisted with manuscript revisions.

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1.8 Figures

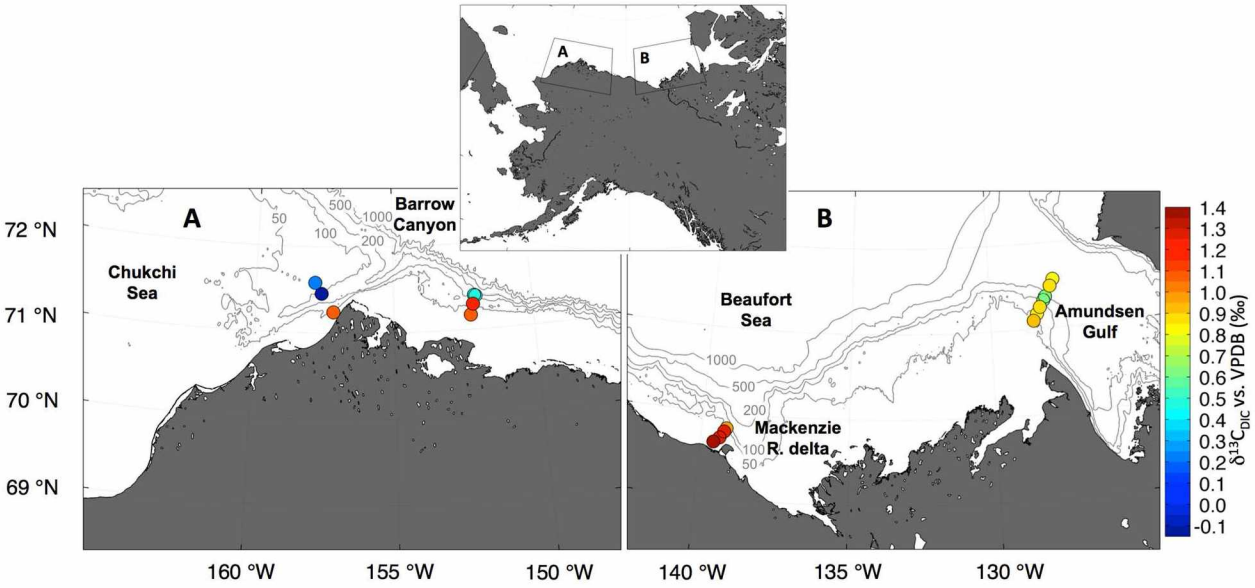

Figure 1.1 $\delta^{13}C_{DIC}$ values (‰) measured from bottom water in the Beaufort and Chukchi seas. Bottom water was collected ~5 m the from sediment-water interface at all sampling locations.

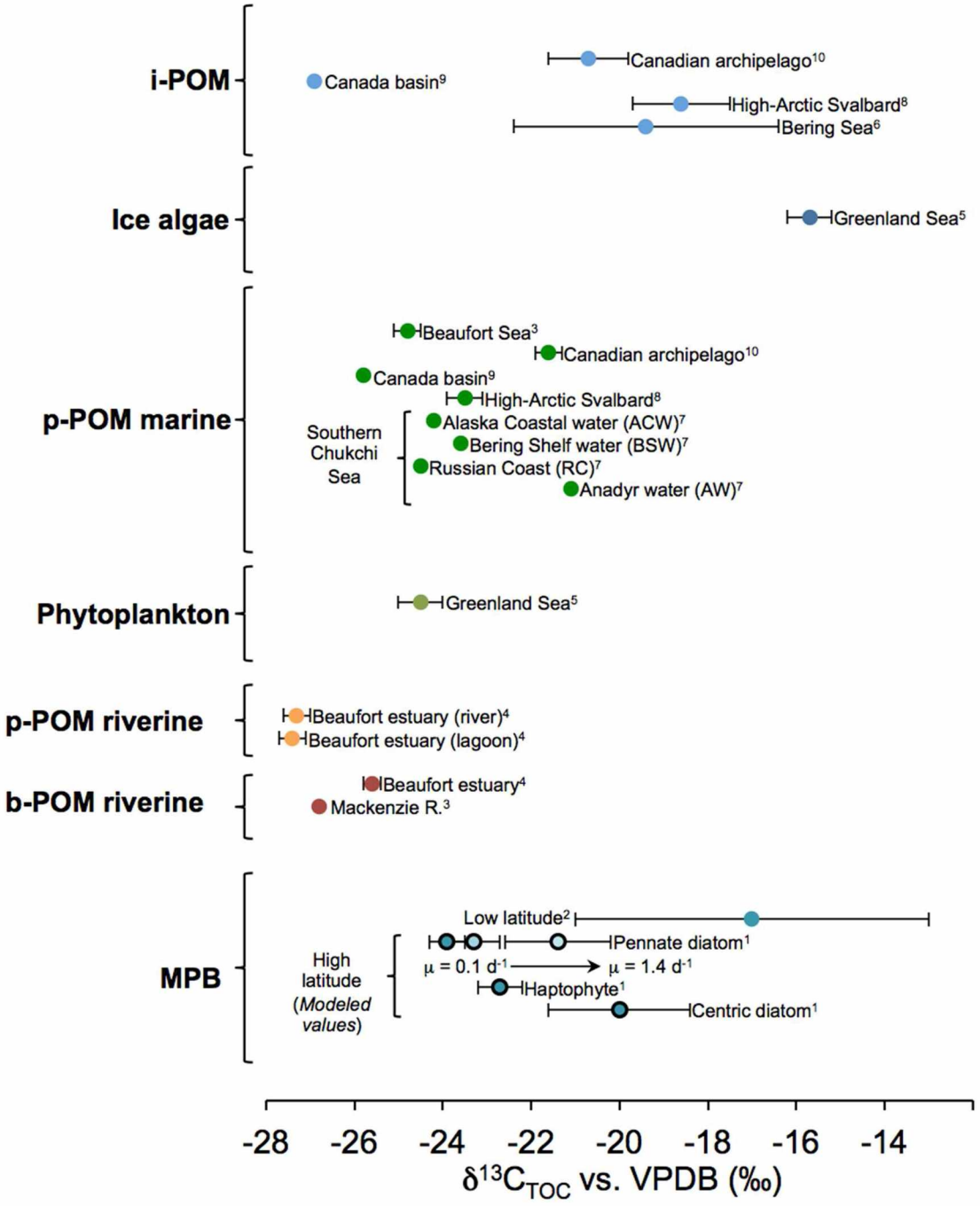

Figure 1.2 $\delta^{13}C$ values (‰) for TOC from primary production sources in the Arctic, sub-Arctic, and low latitude marine environments. Values are mean ± 1 s.d.. Symbols outlined in black are modeled values from the study. Particulate organic matter (POM) measured in ice (i-POM), water (p-POM), and sediment (b-POM) from the marine and riverine environment ([1]this study, [2]France (1995), [3]Naidu et al. (2000), [4]Dunton et al. (2012), [5]McMahon et al. (2006), [6]Wang et al. 2014, [7]Iken et al. (2010), [8]Søreide et al. (2013), [9] Iken et al. (2005), [10]Hobson and Welch (1992)).

1.9 Tables

Table 1.1 Estimates of ε_p and $\delta^{13}C_p$ values (‰) for microphytobenthos. Estimates are based on measured $\delta^{13}C_{DIC}$ values and calculations of $[CO_2]aq$ measured in bottom water in the Beaufort and Chukchi seas.

Station	Water depth (m)	$\delta^{13}C_{DIC}$	$[CO_2]aq$	ε_p[a]	$\delta^{13}C_p$[a]	ε_p[b]	$\delta^{13}C_p$[b]	ε_p[c]	$\delta^{13}C_p$[c]	$\delta^{13}C_p$[c]	$\delta^{13}C_p$[c]
				μ = 0.1 d^{-1}		μ = 0.1 d^{-1}		μ = 0.1 d^{-1}		μ = 0.4 d^{-1}	μ = 1.4 d^{-1}
9	47	1.0	18	19.2	-17.8	23.8	-22.2	25.2	-23.6	-22.7	-20.3
14	112	-0.1	72	24.0	-23.5	24.4	-23.9	25.4	-24.9	-24.7	-20.1
17	66	0.2	43	22.9	-22.2	24.3	-23.5	25.4	-24.5	-24.2	-20.2
23	33	1.1	19	19.6	-18.1	23.9	-22.3	25.2	-23.5	-22.7	-20.1
26	55	1.2	17	19.1	-17.6	23.8	-22.1	25.2	-23.4	-22.6	-19.9
28	165	0.5	33	22.1	-21.2	24.2	-23.2	25.3	-24.3	-23.8	-21.3
48	134	0.5	23	20.7	-19.8	24.0	-23.0	25.3	-24.2	-23.5	-21.6
49	346	0.8	23	20.7	-19.5	24.0	-22.7	25.3	-23.9	-23.2	-19.7
50	284	0.9	27	21.4	-20.1	24.1	-22.7	25.3	-23.8	-23.3	-21.8
52	172	0.6	36	22.4	-21.3	24.2	-23.0	25.4	-24.1	-23.7	-23.0
53	132	0.6	42	22.8	-21.7	24.3	-23.1	25.4	-24.1	-23.8	-21.5
55	75	0.9	28	21.5	-20.2	24.1	-22.7	25.3	-23.8	-23.3	-24.0
57	60	0.8	30	21.8	-20.6	24.1	-22.8	25.3	-23.9	-23.4	-22.6
59	54	0.9	30	21.8	-20.4	24.1	-22.7	25.3	-23.8	-23.3	-21.4
68	50	1.0	27	21.3	-19.9	24.1	-22.6	25.3	-23.7	-23.2	-22.3
69	42	1.3	20	20.0	-18.3	23.9	-22.1	25.2	-23.4	-22.6	-22.3
70	35	1.3	20	20.0	-18.3	23.9	-22.1	25.2	-23.4	-22.6	-21.4
71	28	1.4	21	20.3	-18.5	24.0	-22.1	25.3	-23.3	-22.6	-21.1
mean		0.8	30	21.2	-20.0	24.1	-22.7	25.3	-23.9	-23.3	-21.4
1 standard deviation		0.4	13	1.4	1.6	0.2	0.5	0.1	0.4	0.6	1.2

[a] **centric diatom (*P. glacialis*)** [b] **haptophyte (*E. huxleyi*)** [c] **pennate diatom (*P. tricornutum*)**

Station	Date	Latitude (decimal °)	Longitude (decimal °)	Water depth (m)	Pressure (dB)	Temperature (°C)	Salinity (uncalib)	Chlorophyll (µg/L)	Turbidity (V %)	DIC (µmol/kg)	TA (µmol/kg)	*f*CO2 (µatm)	pCO_2 (µatm)	CO_2 (µmol/kg)	PO_4 (µM)	SiO_4 (µM)	N+N (µM)	NO_2 (µM)	NH_4 (µM)	NO_3 (µM)	DIN (µM)
9	10/11/2012	71.2412	-157.176	47	41.12	3.92	30.43	0.19	43.12	2009	2154	321	322	18	1.05	13.42	0.64	0.10	1.66	0.55	2.30
14	10/11/2012	71.4543	-157.6067	112	104.59	-1.58	33.18	0.14	73.18	2309	2292	1070	1075	72	2.54	44.01	14.25	0.10	5.68	14.15	19.93
17	10/11/2012	71.581	-157.8475	66	58.84	-1.22	32.99	0.14	70.27	2224	n.d.	n.d.	n.d.	n.d.	2.32	34.45	11.97	0.10	3.24	11.86	15.21
23	10/12/2012	71.1697	-152.2485	33	27.17	2.97	30.41	0.18	15.03	2004	2138	328	329	19	1.71	14.71	2.88	0.29	2.81	2.58	5.69
26	10/12/2012	71.2957	-152.1448	55	46.96	2.97	31.44	0.15	68.93	n.d.	n.d.	n.d.	n.d.	n.d.	0.98	10.89	1.44	0.06	1.75	1.38	3.18
28	10/12/2012	71.3978	-152.0835	165	147.82	-0.66	33.85	0.13	80.09	n.d.	n.d.	n.d.	n.d.	n.d.	1.82	23.98	15.42	0.12	1.66	15.30	17.08
48	10/16/2012	71.3883	-152.0355	134	120.34	-0.45	32.85	0.13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
49	10/17/2012	71.5695	-127.6343	346	324.15	0.43	34.82	0.12	88.68	2159	2289	379	380	23	1.20	9.08	17.31	0.00	0.06	17.31	17.37
50	10/17/2012	71.4907	-127.7712	284	268.88	0.04	34.56	0.12	87.28	2175	2281	436	438	27	1.28	11.84	17.03	0.03	0.11	16.99	17.14
52	10/18/2012	71.3697	-127.9687	172	162.20	-1.23	33.48	0.12	88.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
53	10/18/2012	71.3292	-128.0477	132	118.61	-1.46	32.85	0.12	89.91	2204	2250	617	619	42	2.42	24.19	18.84	0.04	0.19	18.81	19.03
55	10/18/2012	71.2533	-128.1923	75	64.78	-0.93	31.91	0.14	79.80	2128	2218	424	426	28	1.73	11.93	6.69	0.14	0.59	6.54	7.28
57	10/18/2012	71.1755	-128.3202	60	52.87	-0.70	31.96	0.14	71.56	2154	2236	463	465	30	0.69	8.68	0.16	0.03	0.19	0.13	0.35
59	10/18/2012	71.0995	-128.458	54	47.25	-0.76	31.87	0.14	50.66	2153	2237	452	454	30	0.86	9.94	0.40	0.08	0.43	0.32	0.84
68	10/19/2012	69.8193	-139.1595	50	44.30	-1.15	32.16	0.13	82.86	2117	2214	398	400	27	1.74	14.25	9.83	0.09	0.20	9.74	10.03
69	10/19/2012	69.781	-139.2278	42	35.93	1.23	28.01	0.18	61.72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
70	10/19/2012	69.7063	-139.3698	35	28.81	1.40	27.73	0.17	61.29	1923	2029	325	326	20	0.84	4.47	0.01	0.01	0.35	0.00	0.35
71	10/19/2012	69.6533	-139.562	28	22.98	0.98	27.70	0.17	42.24	1922	2020	339	341	21	0.94	3.33	0.18	0.09	0.54	0.09	0.72

Appendix 1.1 Physical and chemical properties of bottom water at sampling locations in the Beaufort and Chukchi seas.

Chapter 2 Feeding ecologies of key bivalve and polychaete taxa in the Bering Sea elucidated from fatty acid and compound-specific stable isotope analyses[1]

2.1 Abstract

We investigated differences in algal carbon sources among common bivalve (*Macoma calcarea, Nuculana radiata, and Ennucula tenuis*) and polychaete (*Leitoscoloplos pugettensis* and *Nephtys* spp.) species from the Bering Sea using relative proportions of fatty acids (FA profiles), FAs indicative of distinct organic matter sources (FA markers), and stable carbon isotope values of FA markers. We measured FAs from these species and from surface sediment scrapes collected during March-July in 2009 and 2010. The bivalve species had indistinguishable trophic signatures, as inferred by overlapping FA profiles and $\delta^{13}C$ values for algal marker FAs, and similar proportions of FAs indicative of FA source. FA $\delta^{13}C$ values from the bivalve taxa were most similar to FA $\delta^{13}C$ values from particulate organic matter (POM) from surface sediments. In contrast, $\delta^{13}C$ values for the algal marker eicosapentaenoic acid (20:5n-3) in the polychaetes were higher relative to those from the bivalves and sediment from the same locations (mean difference of 3.6‰), suggesting low direct dietary contributions of benthic POM from surface sediments. *Leitoscoloplos pugettensis*, a head-down deposit-feeding polychaete, had a higher contribution from bacterial sources to its total FA pool relative to the bivalves and to *Nephtys* spp., a predatory polychaete, based on a bacterial FA marker. Distinct FA profiles between the two polychaetes imply different proportional contributions of dietary FA sources, including greater contribution of microbially altered FAs to *L. pugettensis* and greater contribution of ice algal FAs to *Nephtys* spp. Our findings show that benthic invertebrates from distinct feeding groups rely on different proportional contributions of organic matter derived from sympagic, pelagic, and benthic sources in the Bering Sea.

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[1] In revision at Marine Ecology Progress Series as Oxtoby LE, Budge SM, Iken K, O'Brien DM, Wooller MJ "Feeding ecologies of key bivalve and polychaete taxa in the Bering Sea elucidated from fatty acid and compound specific stable isotope analyses".

2.2 Introduction

The Bering Sea is characterized by high benthic biomass (Grebmeier & Cooper 1995, Carmack & Wassman 2006), which sustains populations of high trophic level benthic consumers, such as Pacific walruses, gray whales, bearded seals, and diving ducks (i.e., spectacled eiders) (Lovvorn et al. 2003, Moore et al. 2003, Blanchard et al. 2013), as well as the human communities that rely on these predators (Krupnik & Jolly 2002). Sources of organic matter (OM) in the Arctic marine ecosystem are diverse and can include pelagic, sympagic, and benthic production, along with terrestrial sources (Carmack et al. 2006). However, the degree to which these different sources of OM contribute to supporting these highly productive benthic communities in the Bering Sea is not fully understood.

Annual primary production in seasonally ice-covered seas consists largely of pelagic and under-ice production (Gosselin et al. 1997, Sakshaug 2004, Arrigo et al. 2012). A smaller amount of total annual production is sympagic, originating within the sea ice matrix (Gradinger 2009). The contribution of *in situ* benthic production (including algal and microbial sources) to total annual production is unknown. In the sub-Arctic, spatial and temporal differences in light availability, sea ice cover, and nutrient supply influence the development of pelagic and sympagic algal blooms (Grebmeier & McRoy 1989, Gradinger 2002, Carroll et al. 2008). Physical and biological factors, such as wind mixing, the timing of ice melt, stratification, and pelagic grazing, subsequently affect the extent to which this material is deposited on the seafloor (Grebmeier & Barry 1991 and references therein, Renaud et al. 2007b, Cooper et al. 2009). Seasonal deployment of sediment traps under varying sea ice conditions have shown a wide range of vertical flux rates for a given geographical area with peak carbon flux observed in spring (Grant et al. 2002, Moran et al. 2005, Morata & Renaud 2008).

At the seafloor, benthic invertebrate communities in high latitude marine environments metabolize organic matter, causing sediment oxygen demand to increase and concentration of algal pigments (chlorophyll a and phaeopigments) to decline (Grebmeier & McRoy 1989, Ambrose & Renaud 1995, Piepenburg et al. 1997, Dunton et al. 2005, Renaud et al. 2007b). Sympagic and pelagic particulate OM (defined here as i-POM and p-POM, respectively) are generally considered high quality food sources for benthic consumers (Kanneworff & Christensen 1986, Grebmeier & McRoy 1989, Renaud et al. 2007a), as they contain large amounts of polyunsaturated fatty acids (PUFAs) (Leu et al. 2010, Søreide et al. 2010) that cannot

be synthesized *de novo* by consumer organisms (Parrish 2013). In addition to fresh i-POM and p-POM, benthic consumers have access to a heterogeneous mixture of OM within the sediment (benthic OM or b-POM). B-POM can comprise *in situ* production by microbes (Graf et al. 1982, Meyer-Reil 1983, Boetius & Damm 1998) and microphytobenthos (Matheke & Horner 1974, Karsten et al. 2006, Glud et al. 2009), as well as degraded phytodetritus originating from marine, terrestrial, and freshwater environments (Naidu et al. 1993, Naidu et al. 2000, Pirtle-Levy et al. 2009). Physical and biological processes, including resuspension and burial, bioturbation, and microbial degradation, influence the distribution and chemical composition of b-POM within the sediment (e.g., Clough et al. 1997, Arnosti & Jørgensen 2006, Konovalov et al. 2010, Morata et al. 2011, Teske et al. 2011).

The relative importance of OM sources to benthic invertebrate diets in the Arctic and sub-Arctic has been investigated using stable isotope analysis (Iken et al. 2005, Lovvorn et al. 2005, McTigue & Dunton 2014), because pelagic algae (p-POM), ice-associated algae (i-POM) and b-POM can differ in their stable carbon isotope ratios (expressed as $\delta^{13}C$ values) (e.g., Hobson & Welch 1992, Lovvorn et al. 2005, Søreide et al. 2006). However, stable isotope analyses of benthic consumers have generated a wide range of values that cannot be attributed to anticipated sources (Iken et al. 2005). For example, Lovvorn et al. (2005) and McTigue & Dunton (2014) observed $\delta^{13}C$ values for invertebrate consumers that were consistently higher than $\delta^{13}C$ values from OM sources originating in the water column and in surface sediments. Values were similar to those of i-POM, but i-POM was not available to consumers during (and weeks prior to) sample collection in the aforementioned studies. Consequently, it has been suggested that benthic consumers may rely on phytodetrital POM that remains after microbial degradation (Lovvorn et al. 2005, McTigue & Dunton 2014). The phytodetrital POM that remains could be higher in ^{13}C relative to the OM sources analyzed due to fractionation associated with microbial degradation (Sun et al. 2004). Complementary analytical methods such as fatty acid (FA) analyses can further elucidate contributions of uncharacterized isotopic sources, such as microbially altered OM, to consumer diets.

FA analyses typically include the relative proportions (% of total) of all FAs in a sample (a FA profile) and a relative proportion or sum of individual FAs of particular interest (FA markers); for example, that may be characteristic of diatoms, dinoflagellates, and bacteria (e.g.,

Viso & Marty 1993, Dalsgaard et al. 2003, Parrish 2013). FA profiles and markers are important ecological tools to infer diet composition because they can reveal differences in diet and source partitioning within a food web (e.g., Graham et al. 2014, Wang et al. 2015). Individual FAs can also be analyzed for their $\delta^{13}C$ values, which can differ depending on the source from which the FA was derived. As such, they provide an independent and complementary line of evidence to determine particular source contributions to a consumer or sample (Budge et al. 2008, Wang et al. 2015).

We examined the sources of FAs to benthic consumers to investigate whether organisms assimilate different POM sources in the sub-Arctic benthic environment in the Bering Sea. We used stable isotope analyses of algal marker FAs in concert with FA profiles and various metrics (relative proportions and sums of individual FAs) (e.g., Sun et al. 2007, Budge et al. 2007, 2008, Wang et al. 2015) to investigate resource partitioning among bivalve and polychaete taxa that are abundant in the Bering Sea benthos. We included suspension/surface deposit-feeding bivalves (*Macoma calcarea* (Gmelin, 1791) and *Ennucula tenuis* (Montague 1808)), a subsurface deposit-feeding bivalve (*Nuculana radiata* (Krause 1885)), a head-down deposit-feeding polychaete (*Leitoscoloplos pugettensis* (Pettibone, 1957)) and a predatory scavenging polychaete (*Nephtys* spp.). We hypothesized that FAs would reflect differences in feeding strategies among benthic invertebrate consumers.

2.3 Materials and methods

2.3.1 Study area

The Bering Sea serves as a sub-Arctic transition zone between the temperate North Pacific Ocean and Arctic marine ecosystems of the Chukchi Sea. It consists of a deep basin (> 2000 m) to the west and a shallow continental shelf (< 200 m) to the east. The eastern shelf is separated by oceanographic fronts that subdivide the region into three domains with distinct physical (Coachman 1986) and biogeochemical (Mathis et al. 2010) features, and annual primary production (g C m^{-2} $yr^{-}1$) (Springer et al.1996). These regions are delineated by the 50, 100, and 200 m isobaths, which separate the inner (< 50 m), middle (50-100 m), and outer (> 100-200 m) shelf domains.

The Bering Sea is characterized by the most dramatic seasonal advance and retreat of sea ice of any region in the Arctic or sub-Arctic (Niebauer 1983, Bluhm & Gradinger 2008). Ice formation in the Bering Sea begins as prevailing winds drive ice southward to the shelf break where the storage of deep basin heat prevents further advancement (Niebauer et al. 1999). On average, ~37 % of the Bering Sea is seasonally ice covered (Niebauer 1983). Sea ice minima and maxima in the Arctic generally occur in September and February, respectively. However, sea ice extent is subject to interannual variation due to large-scale ocean-atmosphere interactions, such as the El Niño/Southern Oscillation (ENSO) and the Pacific-North American pattern (PNA) (Niebauer 1988, Stabeno et al. 1999). Additionally, the Aleutian Low pressure system, created by the occurrence of winter storms, drives cooling or warming in the Bering Sea, depending on its strength and position (Niebauer et al. 1999, Stabeno et al. 2001). Observations of September sea ice minima in the Arctic between 1953 and 2006 have shown a 7.8-10.7 % reduction in sea ice extent per decade (Stroeve et al. 2007, 2008). Observational data indicate that sea ice extent may continue to decline at a rate faster than predicted by current modeling efforts (Stroeve et al. 2007).

2.3.2 Sample acquisition

Sediment (n = 82), i-POM (n = 21), p-POM (n = 55), and invertebrate samples (n = 62) were collected opportunistically from the Bering Sea during three research cruises in 2009 and three in 2010 as part of the Bering Sea Ecosystem Study- Bering Sea Integrated Ecosystem Research Program (BEST-BSIERP). In 2009, samples were collected during March and April (cruises HLY0901 and HLY0902, U.S. Coast Guard Cutter *Healy*), followed by sampling during June-July (cruise KNORR195, R/V *Knorr*). In 2010, samples were collected during March (cruise PSEA10-01, USCGC *Polar Sea*), May-June, and June-July (cruises TN249 and TN250, R/V *Thomas G. Thompson*). Data and sample collection occurred each year during times ranging from maximum ice extent to ice-free conditions. Ice extent reached a maximum on February 28th in 2009 and persisted through March (NSIDC 2009). In 2010, maximum ice extent occurred one month later on March 31st (Richter-Menge & Overland 2010). Sampling stations were located south of St. Lawrence Island (Appendix 2.1, Fig. 2.1). Water column depths ranged from ~30 to 500 m.

At each station, a CTD (Seabird 911plus system using dual temperature, conductivity, and oxygen sensors) was used to measure temperature and salinity. Additional information describing sampling stations is provided in Appendix 2.1. A van Veen grab was deployed to collect surface sediment scrapes and invertebrate specimens. Invertebrate specimens were not present at all stations where surface sediment scrapes were collected (Appendix 2.1 and Table 2.1). When available, invertebrate specimens were identified to species with the exception of *Nephtys* spp. All samples, including surface sediment scrapes, were frozen at -20°C until they were freeze-dried for further analyses (described below). I-POM and p-POM sample collection and processing in 2009 and 2010 are described by Wang et al. (2015) and Wang et al. (2014), respectively.

2.3.3 Fatty acid methyl ester transesterification and analysis

Lipids were extracted from sediments and invertebrate specimens using a modified Folch procedure (Folch 1957, Budge et al. 2006). All visible macroinfaunal material was removed from sediment samples prior to sediment lipid extraction. Between 3 and 25 g of freeze-dried sediment was acidified to remove inorganic carbon by soaking overnight in 1N HCl until bubbling ceased. Sediments were rinsed 10-12 times with 30 mL of nanopure water for each rinsing or until the pH of the supernatant became equal to the pH of nanopure water (> 5.5). Multiple specimens from invertebrate taxa (< 5 individuals) were pooled when necessary to obtain sufficient lipid content for FA analyses. Mean lipid content varied among taxa (*L. pugettensis* = 13.6 ± 7.8 mg/100 mg wet weight (ww), *Nephtys* spp. = 2.3 ± 1.2 mg/100 mg ww, *M. calcarea* = 1.7 ± 1.1 mg/100 mg ww, *E. tenuis* = 7.2 ± 8.9 mg/100 mg ww, *N. radiata* = 3.9 ± 2.2 mg/100 mg ww) (see also Appendix 2.2). Shells were removed and invertebrate tissues were homogenized to reduce particle size. Gut contents were not removed prior to lipid extraction.

Lipids were extracted using a solvent mixture of HPLC-grade chloroform, methanol, and de-ionized water in a ratio of 8:4:3. This solvent mixture was added to sediments and invertebrates and left to soak overnight at 4°C. Samples in solvent were then rinsed, centrifuged, and the lipid extract was evaporated under nitrogen in a water bath at 25-30°C. Extracted acyl lipids were then transesterified into their constituent FA methyl esters (FAME) using sulfuric acid (H_2SO_4) as a catalyst.

Relative proportions of individual FAs were determined using gas chromatography as described by Budge et al. (2006). FAME in hexane (1 μL) were introduced via splitless injection into a Perkin Elmer Autosystem II (Perkin Elmer, Boston, MA, USA) gas chromatograph (GC) with flame ionization detector (FID) fitted with a 30 m 0.25□mm i.d. column coated with 50 % cyanopropyl polysiloxane (0.25□mm film thickness; J&W DB-23; Folsom, CA, USA). GC-mass spectrometry (MS) (Thermo Finnigan Polaris Q; Bremen, Germany) was used, applying the same conditions as GCFID, to characterize constituent FAs in an Atlantic menhaden (*Brevoortia tyrannus* (Latrobe, 1802)) oil sample containing FAs common to sediment and invertebrate samples. Retention times for sediment and invertebrate samples were then cross-referenced to those from the menhaden oil profile to identify individual FAs. FAs are described by the nomenclature A:Bn-X, wherein A indicates the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group of a FA. *Iso-* and *antiso-* methyl branched FAs are further identified by lowercase italicized letters (e.g., *i*-15:0, a FA with 15 carbon atoms, 0 double bonds and a methyl branch on the second to last carbon atom in the chain).

We report relative proportions and sums of individual FAs that have been used as markers to identify OM sources (reviewed in Parrish 2013) in benthic invertebrate taxa (Table 1; complete FA datasets are available on request). We removed FAs present at relative proportions less than 0.01 % total and renormalized FA data prior to statistical analysis. We then excluded non-methylene interrupted (NMI) FAs, which are synthesized by a diverse set of benthic invertebrate taxa, including bivalves (Paradis & Ackman 1977, Joseph 1979, 1982, Kawashima 2005, Monroig et al. 2012). As a "benthic" marker, NMI FAs do not provide information on dietary carbon sources for organisms that produce them and may obscure differences among FA profiles of bivalve and non-bivalve taxa in our study. The sum of FAs with branched chains and odd numbers of carbon atoms (*ai*-15:0, *i*-15:0, *ai*-17:0, *i*-17:0) is considered to be a composite bacterial marker (reviewed in Parrish 2013). Relative proportions of 16:4n-1, 16:1n-7, 20:5n-3, and 22:6n-3 are considered to be algal indicators (e.g., Dalsgaard et al. 2003, Kelly & Scheibling 2012). Accordingly, the sum of PUFAs can be an indicative of high algal contributions.

2.3.4 Stable carbon isotope analysis of individual FAME

To measure the $\delta^{13}C$ values of individual FAs, 1 µL of FAME from each sample in hexane was injected on to a GC column connected to an isotope ratio mass spectrometer (IRMS - Thermo Finnigan Delta V) via a combustion interface (IsoLink; www.isolink.com) at the Alaska Stable Isotope Facility (ASIF). Sample concentration of FAME in hexane was optimized to produce a voltage of 500-3000 mV for 20:5n-3. The GC column, temperature program, and mode of injection were the same as the GC-FID analyses (described above). A FAME standard consisting of 16:0 and 18:0 (Nu-Chek Prep, Inc.; Elysian MN) was run between every 8 samples (n = 20) to track analytical error, which was < 0.1‰ and < 0.2‰, respectively (expressed as 1 SD of 16:0 and 18:0).

Stable carbon isotope ratios of samples are expressed relative to the ratios of the international standard Vienna Pee Dee Belemnite (VPDB) using conventional delta (δ) notation in parts per thousand (‰) according to the following equation (Eq. 2.1):

$$(2.1) \quad \delta X = [(R_{standard}/R_{sample})-1]*1000$$

where $\delta X = \delta^{13}C$ value and R is the ratio of $^{13}C/^{12}C$. To calibrate the $\delta^{13}C$ values, we used a standard mixture containing eight calibrated *n*-alkanoic acid esters (Mixture F8, Indiana University Stable Isotope Reference Materials), where r^2 of the known versus expected relationship was > 0.99. To account for the carbon added during transesterification, we corrected $\delta^{13}C_{FA}$ values using the following equation (Eq. 2.2):

$$(2.2) \quad \delta^{13}C_{FA} = [(n+1)(\delta^{13}C_{FAME})-(\delta^{13}C_{methanol})]/n$$

where $\delta^{13}C_{FA}$ is the adjusted value of the FA of interest, *n* is the number of its carbon atoms, $\delta^{13}C_{FAME}$ is the F8-mixture calibrated value of the FAME, and $\delta^{13}C_{methanol}$ is the stable isotope composition of the carbon contributed by the methanol (Abrajano et al. 1994). $\delta^{13}C_{methanol}$ ($\delta^{13}C_{methanol}$ = -49‰) was calculated by subtracting the $\delta^{13}C$ value of esterified C16 and C18 standards from the corresponding $\delta^{13}C$ values of their free FAs (Wang et al. 2014).

2.3.5 Data analysis

Due to the opportunistic nature of sample collection, we did not have sufficient sample replicates to test variability associated with year, geography, or ice condition (sample sizes included in Table 2.1). Therefore, we pooled data by taxon and performed univariate and multivariate statistical techniques to determine differences among invertebrate species. FA data were standardized to 100 % and transformed using a log(1+X) function prior to multivariate analyses (Budge et al. 2006) because proportional data are rarely normally distributed. Bray-Curtis similarity matrices were constructed on FA data for those FAs present at proportions > 0.1 % (n = 71 FAs). Permutational multivariate analysis of variance (one-factor PERMANOVA with pairwise comparison) (Anderson 2001) was performed using PRIMER-6 (Primer-E Ltd) to describe variation among FA profiles of individual species (Table 2.2). Statistical significance of pairwise differences between FA profiles was determined based on a 99 % confidence level (α = 0.01) adjusted to maintain a familywise error rate using a Bonferroni correction (Primer-E Ltd) (Table 2.2). Similarity percentages routines (SIMPER) were run to identify the FAs that contributed most to differences in the relative proportions of FAs among samples. Non-metric scaling (nMDS) plots were generated to present differences between FA profiles among species and sources of POM (Fig. 2.2) and among individual FAs among species (Fig. 2.3).

Most FA markers (relative proportions and sums) and $\delta^{13}C$ values of FAs violated assumptions for parametric statistics so we used a Kruskal-Wallis one-factor analysis of variance (ANOVA) (R version 3.2.0) and a Mann-Whitney *U*-test to test for pairwise differences among benthic invertebrate species (Tables 2.1, 2.3). Statistical significance was determined based on a 99 % confidence level (α = 0.01) corrected for the number of comparisons made using a Bonferroni correction (R version 3.2.0). $\delta^{13}C$ values (mean ± SD) of non-marker FAs common to the five invertebrate taxa (16:0, 18:0, 18:1n-9, 18:1n-7, and 20:1n-7) are provided in Appendix 2.4.

We performed a series of Bayesian multi-source stable isotope mixing models (SIAR, Parnell et al. 2010) using 16:1n-7, 20:5n-3, and 22:6n-3 to estimate the proportional contributions of i-POM, p-POM, and b-POM (specifically the fraction of b-POM in surface sediment scrapes) to bivalve taxa and to *Nephtys* spp. (Table 2.4). We did not include *L. pugettensis* because its distinct isotopic composition in combination with FA analyses indicated

that it likely sources FAs from a fraction of b-POM that we did not sample (specifically microbially altered phytodetrital POM that may be abundant in sediment located below the top centimeter) (see Discussion). $\delta^{13}C$ values for 16:1n-7, 20:5n-3, and 22:6n-3 from surface sediment b-POM fell between values from i-POM and p-POM, which could imply that b-POM consists of an equal mixture of FAs from i-POM and p-POM. However, we selected a three source mixing model that includes b-POM as a unique source based on differences in FA profiles (Fig. 2.2) and relative proportions of FAs indicative of carbon source among POM sources (Table 2.5, see Appendix 2.3 for the FA composition of POM sources). We further discuss the implications of including b-POM as a unique FA source to our mixing model results in the Discussion.

We ran each Bayesian mixing model with and without concentration dependencies (e.g., Wang et al. 2015), which adjust model estimates to account for the relative proportions of FAs (% total) in each source (Table 2.5). However, we report only the results from the concentration-dependent model because our findings were the same for both models. We assumed a FA trophic isotope enrichment factor of zero in all models as in Wang et al. (2015) (see also Discussion). There have not been studies on trophic enrichment at the molecular level in marine bivalves or polychaetes to provide trophic enrichment factor estimates. However, research on trophic enrichment factors for Steller's eiders and spectacled eiders (Budge et al. 2011) showed no isotopic differences in 20:5n-3 and 22:6n-3 from dietary sources and those from adipose tissue (Budge et al. 2011). The mean discrimination factor for 16:1, was ~+1‰, suggesting that trophic enrichment factors may vary minimally for individual FAs in marine organisms (Budge et al. 2011).

2.4 Results

2.4.1 Fatty acid profiles and markers

FA profiles significantly differed between *L. pugettensis* and *Nephtys* spp. and between all polychaete-bivalve species pairings, but not among bivalve species (1-factor PERMANOVA with pairwise comparison) (see Table 2.2 for *P* values). FA profiles between *L. pugettensis* and *Nephtys* spp. were 32 % dissimilar, with elevated relative proportions of bacterial markers (*ai*-15:0, *i*-15:0) in *L. pugettensis* contributing most to the dissimilarity (SIMPER) (Fig. 2.3). Higher relative proportions of 22:1n-11 and 16:1n-7, and a lower average relative proportion of 22:6n-3

in *L. pugettensis* relative to *Nephtys* spp., also differentiated the FA profiles of the polychaete taxa (SIMPER) (Fig. 2.3). These FAs (*i*-15:0, *i*-15:0, 22:6n-3, 22:1n-11) also contributed most to the dissimilarities among *L. pugettensis* and the bivalve taxa. FAs that contributed most to differences in FA profiles among *Nephtys* spp. and the bivalve taxa were 20:2n-6, 20:3n-3, 22:4n-6, and 18:1n-13, all of which were higher in *Nephtys* spp. (SIMPER).

Leitoscoloplos pugettensis had the highest mean proportion of the composite bacterial marker (sum of the relative proportions of *iso*- and *anteiso*- 15:0 and 17:0; 2.2 ± 1.5 %) (Kruskal-Wallis one-factor ANOVA $p < 0.001$, Mann-Whitney *U*-test pairwise comparison) (Fig. 2.4). The relative proportion of PUFAs was highest in *Nephtys* spp. (47.3 ± 7.0 %) and lowest in *L. pugettensis* (26.7 ± 8.7 %); bivalves had intermediate levels, ranging from 31.6 ± 8.1 % (*E. tenuis*) to 43.2 ± 6.0 % (*N. radiata*) (Mann-Whitney *U*-test pairwise comparison, $p < 0.01$) (Table 2.1). The proportion of 20:5n-3 was high in all taxa (ranging from 15.8 % total in *L. pugettensis* to 23.5 % in *Nephtys* spp.) (Fig. 2.3). The relative proportion of 22:6n-3 was also considerable in all taxa (4.4 % in *E. tenuis* to 13.5 % in *N. radiata*), with the exception of *L. pugettensis* (1.2 ± 0.6 %, mean ± 1SD) (Fig. 2.3).

2.4.2 Stable carbon isotope values of fatty acids

$\delta^{13}C$ values for algal marker FAs differed among the benthic invertebrate taxa investigated (16:1n-7, 20:5n-3, and 22:6n-3) (Kruskal-Wallis one-factor ANOVA, $p < 0.01$) (Table 2.3, Fig. 2.5). *Nephtys* spp. had the highest $\delta^{13}C$ values for algal marker FAs, which ranged from -23.8 ± 1.6‰ for 20:5n-3 to -23.0 ± 1.5‰ for 22:6n-3 (Table 2.3, Fig. 2.5). $\delta^{13}C$ values for FAs from *L. pugettensis* were similarly high (e.g., -24.9 ± 1.4‰ for 20:5n-3). $\delta^{13}C$ values of individual FAs from the three bivalve species were not significantly different (Mann-Whitney *U*-test pairwise comparison, $p > 0.01$) (Table 2.3, Fig. 2.5).

Mean $\delta^{13}C$ values for 16:1n-7 and 20:5n-3 from b-POM (-26.7 ± 1.0‰ and -26.9 ± 1.7‰, respectively) fell between values for i-POM and p-POM (Fig. 2.5). The mean $\delta^{13}C$ value for 22:6n-3 from b-POM (-27.2 ± 2.1‰) was very similar to p-POM (-27.9 ± 2.4‰), though still lower than i-POM (-24.2 ± 3.2‰). $\delta^{13}C$ values of 20:5n-3, an algal indicator, from samples of invertebrates and b-POM (surface sediment) collected concurrently overlapped for all bivalve taxa (Fig. 2.6). In contrast, the offset between $\delta^{13}C$ values of 20:5n-3 from polychaete tissue

samples and surface sediment b-POM was significantly greater (~3.6‰ higher than b-POM on average) relative to the bivalves (Kruskal-Wallis one-factor ANOVA $p < 0.0001$; Mann-Whitney *U*-test pairwise comparison) (Fig. 2.6).

Based on different combinations of indicator FAs (16:1n-7, 20:5n-3, and 22:6n-3) and their $\delta^{13}C$ values in SIAR, we produced a range of estimates for proportional contributions of b-POM, p-POM, and i-POM FAs (mean %) to the diets of four invertebrate taxa (*L. pugettensis* excluded) (Table 2.4). FAs from b-POM contributed most to bivalve FA pools, with mean estimates ranging from 45-62 % for *M. calcarea*, 42-66 % for *E. tenuis*, and 45-50 % for *N. radiata*, depending on the FAs selected for the model (Table 2.4). I-POM was the largest contributor of FAs to *Nephtys* spp., with mean contributions ranging from 74-79 %.

2.5 Discussion

FA analyses indicated differences in diet for the bivalves compared with the polychaete taxa and between the two polychaete taxa examined. The three bivalve species had overlapping FA profiles, indicating that they obtained FAs from the same source or mixture of sources. The $\delta^{13}C$ values of marker FAs from bivalves were indistinguishable from sediment (b-POM) and exhibited little variability, despite pooling samples by taxon across varying sampling conditions. FA profiles from polychaete taxa were distinct from bivalves and between polychaete taxa, implying unique FA sourcing likely as a result of different feeding strategies. $\delta^{13}C$ values of algal FA markers (16:1n-7, 20:5n-3, and 22:6n-3) in both polychaete taxa were high relative to those in bivalves, indicating reliance on OM sources other than surface sediment b-POM and p-POM. In *L. pugettensis*, an elevated bacterial marker suggests that the relatively high $\delta^{13}C$ values may be attributed to a microbially altered phytodetrital FA pool. In contrast, *Nephtys* spp. does not show an elevated bacterial marker; therefore, we posit that high $\delta^{13}C$ values for algal marker FAs in *Nephtys* spp. are derived from i-POM. Overall, our findings support our initial hypothesis that FA sources would vary by feeding strategy. The one exception was *N. radiata*, a subsurface deposit-feeding bivalve, whose FAs were indistinguishable from the suspension/surface deposit-feeding bivalves we examined.

2.5.1 Sources of FAs to sediments and bivalve taxa

Based on the $\delta^{13}C$ values of algal markers, *M. calcarea*, *E. tenuis*, and *N. radiata* appear to consistently assimilate algal FAs from a mixture of sources, including b-POM (from surface sediment), i-POM, and p-POM. Estimates of FA contributions to bivalve diets indicated that a majority of FAs originated from b-POM (> 40 %), relative to i-POM (< 30 %) and p-POM (< 30 %). When individual bivalves from each species were paired with surface sediment scrapes collected concurrently at the same location, there were no differences between $\delta^{13}C$ values for 20:5n-3, indicating that these bivalve species likely sourced OM from the surface sediments they inhabited. However, we note that the $\delta^{13}C$ values for marker FAs (16:1n-7, 20:5n-3, and 22:6n-3) from b-POM from surface sediments fell between those from i-POM and p-POM, allowing for the possibility that b-POM could be a mix of these two sources. If marker FAs from b-POM are a mixture of diagenetically unaltered FAs originating from i-POM and p-POM sources then our mixing model results would be an underestimation of the relative importance of pelagic and sympagic FAs to bivalve diets. While we cannot conclusively determine the fractions of b-POM that originate from fresh or diagenetically-altered i-POM and p-POM, or from *in situ* production (e.g., microphytobenthos), evidence from our supporting FA data (FA profiles and relative proportions of individual FAs) indicates that i-POM, p-POM, and b-POM FA sources are unique. Furthermore, a distinct advantage of treating these three POM sources separately is that we can account for indirect assimilation of p-POM and i-POM sources via the benthos (b-POM).

Previous research on bivalves, including *M. calcarea*, *E. tenuis*, and *N. radiata*, has indicated that bivalves consume i-POM, p-POM, and b-POM based on compound-specific stable isotope analyses (Sun et al. 2007, 2009) and stable isotope analyses of total organic carbon (TOC) (Weems et al. 2012, Tu et al. 2015). Additional controlled feeding studies are necessary to resolve the question of whether bivalves preferentially forage for or selectively assimilate particular sources, such as i-POM, because there is evidence both to support (McMahon et al. 2006, Sun et al. 2007) and refute (Sun et al. 2009) that claim. Our findings indicate that *N. radiata*, *M. calcarea*, and *E. tenuis* consume FAs that are available in surface sediment irrespective of source and feeding strategy.

2.5.2 Sources of FAs to polychaete taxa

We attribute the relatively high $\delta^{13}C$ values for 20:5n-3 in *L. pugettensis* to a fraction of b-POM located below surface sediments that was not sampled in this study (and thus was not included in our SIAR model) containing microbially altered phytodetrital FAs. Feeding behavior documented in *L. pugettensis* at lower latitudes describes the genus as conveyor-belt feeders that rely on OM sources near the redoxocline, a zone of microbial productivity and diversity located below surface sediments (Bianchi 1988). We observed a significantly higher proportion of bacterial marker in *L. pugettensis* relative to all other invertebrate consumers. Additionally, *L. pugettensis* (and also *Nephtys* spp.) had $\delta^{13}C$ values for 20:5n-3 that were much higher than those from surface sediment scrapes collected at the same location.

Based on FA markers, we are confident that the diet of *L. pugettensis* includes contributions of microbial biomass, but we can only speculate as to why FAs from a microbial pool may have a distinct isotopic composition. Although research on isotopic fractionation of FAs in marine sediments is lacking, there are studies documenting increases in $\delta^{13}C$ values for FAs from algae as microbial degradation progresses in seawater (Sun et al. 2004, Pan et al. 2014). The degree to which a FA pool becomes enriched in ^{13}C over time is dependent on the stage of decomposition, the FAs in question, and whether the environment is oxic or anoxic (Sun et al. 2004). The proposed mechanism by which FA pools become isotopically enriched in ^{13}C relative to the source as a result of microbial degradation is based on a kinetic isotope effect associated with acetyl-CoA during FA biosynthesis (DeNiro & Epstein 1977, Monson & Hayes 1982). Assuming there is a kinetic isotope effect associated with the reverse reaction, then FAs within a given pool that have isotopically light carbon in the carboxyl group will selectively be subject to decarboxylation first (Sun et al. 2004). As a result, the FA pool that remains would theoretically consist of FAs with elevated ^{13}C in the carboxyl group and would become isotopically enriched in ^{13}C. We hypothesize that microbes present in deeper sediment layers catabolize 20:5n-3 from phytodetritus and preferentially catabolize 20:5n-3 that contains a higher ratio of ^{12}C. When microbes catabolize isotopically lighter 20:5n-3, they leave behind a pool of 20:5n-3 that is isotopically heavier relative to the original source. *Leitoscoloplos pugettensis* would then theoretically consume the microbes and the 20:5n-3 remaining after microbial degradation of phytodetritus as it is feeding and incorporate this signature into its tissues.

In recent decades, researchers have isolated a wide range of bacteria capable of producing long chain PUFAs, such as 20:5n-3 and 22:6n-3 (Nichols et al. 1992, Bowman et al. 1997, Skerratt et al. 2002, Gentile et al. 2003, Fang et al. 2006, Freese et al. 2009, Shulse & Allen 2011). In contrast to microbial degradation of algal PUFAs, isotopic fractionation during bacterial synthesis of long chain PUFAs tends to result in lower $\delta^{13}C$ values for PUFAs relative to the carbon source (Teece et al. 1999, Fang et al. 2006). The level of long-chain PUFA production and the degree to which fractionation occurs can vary depending on environmental conditions such as increases in pressure (Fang et al. 2006). We posit that microbial synthesis of long chain PUFAs is negligible due to the abundance of 20:5n-3 and 22:6n-3 originating from algal blooms in the water column (p-POM), sea ice (i-POM), and potentially microphytobenthos (b-POM). As a result, we hypothesize that fractionation associated with microbial synthesis of PUFAs has little influence on the diets of *L. pugettensis* or other benthic invertebrates living in a shallow and productive shelf environment.

In contrast to *L. pugettensis*, the bacterial FA contributions to *Nephtys* spp. were low, which makes it unlikely that the relatively high $\delta^{13}C$ values for 20:5n-3 in this organism came from a microbially altered phytodetrital 20:5n-3 pool. *Nephtys* spp. is a mobile predator-scavenger (Fauchald & Jumars 1979). As such, it would integrate FAs from a diverse set of organisms living in a broader geographic range relative to the other consumers in this study. Based on stable isotope analyses of TOC and nitrogen, *Nephtys* spp. is known to feed at high trophic levels (TL) (up to 3.5 TL) (Iken et al. 2010). Because we have no evidence to assume trophic fractionation to be > 0, we attribute the relatively high $\delta^{13}C$ values in *Nephtys* spp. to i-POM, a FA source in the environment with relatively high $\delta^{13}C$ values compared with other sources (e.g., p-POM and microphytobenthos) (Wang et al. 2014, Oxtoby et al. 2016). Our multi-source mixing model estimates for *Nephtys* spp. indicate that the highest FA contributions originated from i-POM (> 70 %). We hypothesize that *Nephtys* spp. obtains i-POM-derived FAs indirectly via two possible mechanisms. *Nephtys* spp. may be feeding most actively after ice algal bloom material is deposited and consumed by its prey. Alternatively, *Nephtys* spp. may favor consumption of organisms that selectively assimilate i-POM (Sun et al. 2007).

Future research should aim to advance our understanding of the importance of microbially altered and ice algal-derived OM to benthic organisms through more comprehensive

sampling and analyses of additional proxies of diet. For example, measurements of IP_{25}, a low-lability branched isoprenoid that is unique to ice algae (Belt & Müller 2013) would help constrain contributions from i-POM (Brown & Belt 2012). Analyses of ATP concentrations could be used to quantify microbial biomass (Mincks et al. 2005) in stratified sediment samples, to help characterize OM sources potentially available to infaunal organisms, such as *L. pugettensis*, that feed and reside deeper in the sediment.

2.6 Conclusions

In conclusion, assimilation routes for FAs from sympagic, pelagic, and benthic production differ among the benthic invertebrate taxa we investigated from the Bering Sea. Evidence from FA profiles, $\delta^{13}C$ values for algal marker FAs, and relative proportions of individual FAs indicated that bivalve taxa were consuming a similar mixture of FAs available in surface sediment irrespective of POM source. In contrast, *Nephtys* spp. and *L. pugettensis* did not appear to rely on surface sediment b-POM, even indirectly. Our evidence indicated that *Nephtys* spp. consumed a high proportion of biomass deriving from ice algae, whereas *L. pugettensis* appeared to consume microbially altered phytodetrital sources within subsurface sediment horizons, based on high bacterial FA biomarkers and $\delta^{13}C$ values that were outside the range of measured sources. It appears that benthic invertebrates occupy distinct dietary niches that may result from unique feeding strategies and may lead to reduced interspecific competition among benthic consumers.

2.7 Acknowledgments

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2.9 Tables

Table 2.1 Select fatty acid proportions (% total) and sums from benthic invertebrate taxa. Values are means ± 1SD, n = sample size, individuals pooled by taxon. Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. (PUFAs = polyunsaturated FAs, MUFAs = monunsaturates, SFAs = saturates, Bac = odd branched chain FAs.)

	L. pugettensis	*Nephtys* spp.	*M. calcarea*	*E. tenuis*	*N. radiata*
n	12	16	9	15	10
12:0	0.8 ± 0.6	0.6 ± 0.8	1.5 ± 1.7	1.6 ± 1.7	1.4 ± 1.5
14:0	1.7 ± 0.8	0.6 ± 0.3	2.4 ± 1.0	1.7 ± 1.6	1.8 ± 0.5
i-15:0	0.4 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
ai-15:0	0.8 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
16:0	10.5 ± 2.0	11.2 ± 2.5	12.8 ± 0.8	15.1 ± 3.1	11.2 ± 0.8
16:1n-7	24.2 ± 10.7	3.6 ± 2.2	15.2 ± 11.3	14.8 ± 12.1	8.0 ± 6.6
i-17:0	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.5 ± 0.2
ai-17:0	0.4 ± 0.5	0.2 ± 0.3	0.4 ± 0.5	0.2 ± 0.4	0.3 ± 0.4
16:4n-1	0.2 ± 0.1	0.1 ± 0.2	0.2 ± 0.2	0.4 ± 0.4	0.2 ± 0.3
18:0	5.5 ± 1.4	5.7 ± 1.5	5.6 ± 2.8	5.3 ± 3.0	5.8 ± 1.7
18:1n-9	2.2 ± 1.9	4.9 ± 5.4	2.7 ± 1.2	2.8 ± 1.5	1.9 ± 1.1
18:1n-7	7.2 ± 2.1	6.0 ± 1.2	2.8 ± 1.4	6.5 ± 3.3	6.7 ± 3.1
18:2n-6	1.2 ± 1.4	1.1 ± 1.4	0.9 ± 0.7	1.2 ± 0.7	1.0 ± 0.8
18:2n-4	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
18:3n-3	0.4 ± 0.2	0.3 ± 0.4	0.1 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
18:4n-3	0.4 ± 0.2	0.2 ± 0.1	0.8 ± 0.4	0.8 ± 0.4	0.7 ± 0.2
20:1n-11	4.0 ± 1.4	4.5 ± 1.5	4.4 ± 2.6	6.0 ± 3.4	6.4 ± 1.4
20:1n-9	0.6 ± 0.3	1.5 ± 0.4	0.9 ± 0.4	0.5 ± 0.3	0.5 ± 0.2
20:1n-7	2.2 ± 0.7	3.3 ± 0.8	4.7 ± 0.5	4.6 ± 1.6	2.4 ± 0.6
20:2n-9	0.2 ± 0.1	0.5 ± 0.3	0.5 ± 0.3	0.3 ± 0.3	0.1 ± 0.1
20:2n-6	0.2 ± 0.1	0.7 ± 0.3	0.2 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
20:4n-6	1.9 ± 0.7	2.5 ± 0.9	3.0 ± 1.5	3.7 ± 2.2	2.3 ± 0.6
20:5n-3	15.8 ± 6.5	23.5 ± 4.5	21.2 ± 3.1	15.6 ± 3.8	17.5 ± 1.5
22:1n-11	0.9 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
22:1n-9	0.3 ± 0.1	0.2 ± 0.3	0.5 ± 0.4	0.6 ± 0.4	0.6 ± 0.4
21:5n-3	0.2 ± 0.1	0.4 ± 0.1	1.2 ± 0.8	0.5 ± 0.3	1.2 ± 0.4
22:4n-6	0.8 ± 0.5	1.5 ± 0.5	0.2 ± 0.1	0.2 ± 0.2	0.3 ± 0.4
22:5n-6	0.2 ± 0.1	0.5 ± 0.2	0.5 ± 0.3	0.7 ± 0.5	1.0 ± 0.3
22:5n-3	1.8 ± 0.8	3.1 ± 0.9	2.7 ± 1.7	1.4 ± 1.0	3.3 ± 1.1
22:6n-3	1.2 ± 0.6	11.4 ± 3.1	6.2 ± 2.9	4.4 ± 2.3	13.5 ± 4.4
ΣPUFAs	26.7 ± 8.7^{a}	47.3 ± 7.0^{bd}	39.6 ± 5.5^{abc}	31.6 ± 8.1^{a}	43.2 ± 6.0^{cd}
ΣMUFAs	45.2 ± 9.0^{a}	28.0 ± 6.3^{b}	34.2 ± 8.9^{ab}	38.9 ± 11.2^{ab}	29.8 ± 8.5^{b}
ΣSFAs	21.1 ± 3.2^{ab}	20.8 ± 4.2^{a}	24.1 ± 4.1^{ab}	25.6 ± 4.1^{b}	22.1 ± 3.5^{ab}
Σ Bac	2.2 ± 1.5^{a}	0.5 ± 0.3^{b}	0.8 ± 0.5^{b}	0.7 ± 0.6^{b}	0.9 ± 0.6^{b}

Table 2.2 *P* values from pairwise comparisons of fatty acid profiles of benthic invertebrate taxa. Statistically significant differences (in bold) between polychaete taxa (*L. pugettensis*, *Nephtys* spp.) and bivalve taxa (*M. calcarea*, *E. tenuis*, *N. radiata*) are based on 1-way permutational multivariate analysis of variance (taxon as factor) and a Bonferroni adjustment for pairwise comparisons ($\alpha = 0.01$).

Taxon	*P*	Permutations
L. pugettensis - *Nephtys* spp.	**0.01**	998
M calcarea - *E. tenuis*	> 0.01	997
E. tenuis - *N. radiata*	> 0.01	998
M. calcarea - *N. radiata*	> 0.01	992
Nephtys spp. - *M. calcarea*	**0.01**	998
Nephtys spp. - *N. radiata*	**0.01**	999
Nephtys spp. - *E. tenuis*	**0.01**	997
L. pugettensis - *M. calcarea*	**0.01**	994
L. pugettensis - *E. tenuis*	**0.01**	999
L. pugettensis - *N. radiata*	**0.01**	998

Table 2.3 Mean $\delta^{13}C$ values (‰) of algal marker fatty acids from benthic invertebrate taxa. Algal marker fatty acids are 16:1n-7, 20:5n-3, and 22:6n-3 (means ± 1SD, individuals pooled by taxon, n.d. = no data). Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. Letters a-d indicate significant differences among taxa (Kruskal-Wallis one-factor ANOVA, $p < 0.0001$, Mann-Whitney *U*-test with a Bonferroni adjustment for pairwise comparisons, $\alpha = 0.01$).

	L. pugettensis	***Nephtys*** spp.	***M. calcarea***	***E. tenuis***	***N. radiata***
16:1n-7	-26.7 ± 2.6^{bc}	-23.6 ± 2.5^{ab}	-26.9 ± 0.7^{c}	-26.7 ± 1.1^{c}	-27.3 ± 1.3^{c}
20:5n-3	-24.9 ± 1.4^{a}	-23.8 ± 1.6^{a}	-27.5 ± 0.5^{b}	-26.8 ± 0.9^{b}	-27.2 ± 1.0^{b}
22:6n-3	n.d.	-23.0 ± 1.5^{a}	-25.7 ± 0.7^{b}	-25.6 ± 1.0^{b}	-25.9 ± 0.6^{b}

Table 2.4 Estimates of the proportional contributions (%) of sympagic, pelagic, and benthic particulate organic matter to benthic invertebrate diets. Values are based on four SIAR stable isotope mixing models (means (95 % credibility intervals)) run with concentration dependencies (Table 2.5) and a trophic isotope enrichment factor of zero. Particulate organic matter (POM) includes sympagic (ice) (i-POM), pelagic (p-POM), and benthic (b-POM) sources. Polychaete taxa include *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. *L. pugettensis* was excluded from the mixing models because our evidence indicated that it likely sources FAs from a fraction of b-POM that we did not sample (specifically sediment located below the top centimeter).

i-POM	***Nephtys*** spp.	***M. calcarea***	***E. tenuis***	***N. radiata***
16:1n-7, 20:5n-3, 22:6n-3	79 (62-96)	18 (3-34)	28 (9-45)	24 (7-40)
16:1n-7, 20:5n-3	74 (47-97)	12 (0-26)	18 (1-36)	21 (2-38)
16:1n-7, 22:6n-3	78 (60-95)	25 (7-42)	32 (13-50)	28 (11-46)
20:5n-3, 22:6n-3	74 (51-96)	26 (4-47)	32 (10-54)	26 (7-47)
p-POM				
16:1n-7, 20:5n-3, 22:6n-3	4 (0-10)	23 (5-42)	19 (1-38)	26 (6-45)
16:1n-7, 20:5n-3	5 (0-16)	26 (8-44)	16 (0-35)	32 (9-52)
16:1n-7, 22:6n-3	5 (0-12)	21 (2-40)	19 (1-39)	26 (5-46)
20:5n-3, 22:6n-3	4 (0-11)	29 (5-52)	25 (2-48)	24 (4-46)
b-POM				
16:1n-7, 20:5n-3, 22:6n-3	17 (0-34)	59 (32-85)	53 (24-84)	50 (22-81)
16:1n-7, 20:5n-3	20 (0-46)	62 (35-87)	66 (33-95)	47 (17-83)
16:1n-7, 22:6n-3	17 (0-35)	54 (27-83)	49 (20-80)	45 (18-76)
20:5n-3, 22:6n-3	22 (0-45)	45 (10-81)	42 (5-78)	49 (14-84)

Table 2.5 Relative proportions of fatty acids (% total) for sympagic, pelagic and benthic particulate organic matter for concentration-dependent stable isotope mixing models. Values are means ± 1SD, with samples pooled by algal source. Particulate organic matter (POM) includes sympagic (ice) (i-POM), pelagic (p-POM), and benthic (b-POM) sources.

Source	**16:1n-7**	**20:5n-3**	**22:6n-3**
i-POM	15.7 ± 6.7	20.1 ± 6.8	2.3 ± 0.8
p-POM	16.2 ± 12.9	11.7 ± 7.0	4.9 ± 3.2
b-POM	27.8 ± 16.1	3.5 ± 2.4	1.0 ± 0.8

2.10 Figures

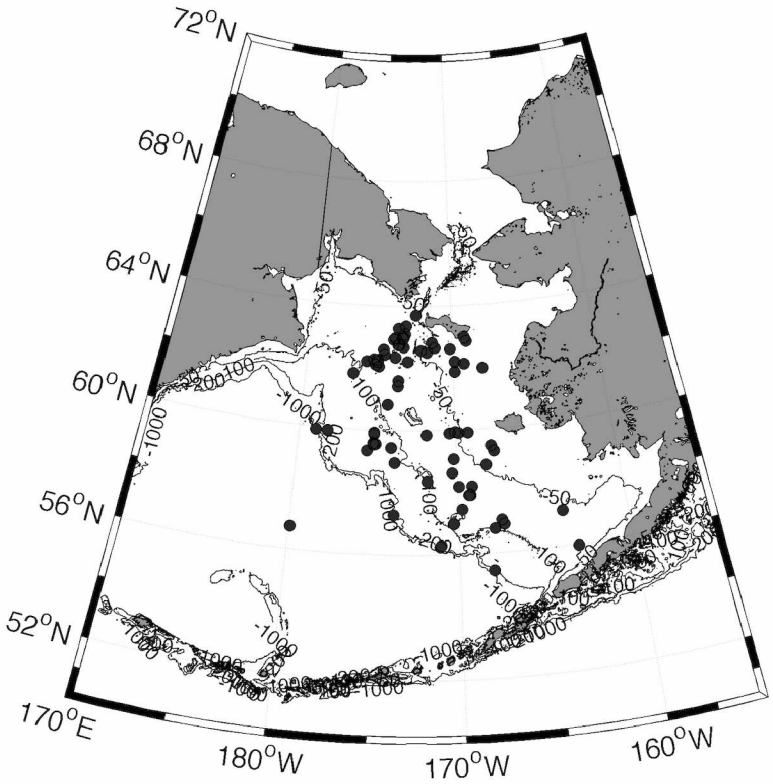

Figure 2.1 Sampling locations for surface sediment scrapes and benthic invertebrate taxa in the Bering Sea. Information on sampling stations is given in Appendix 1.1.

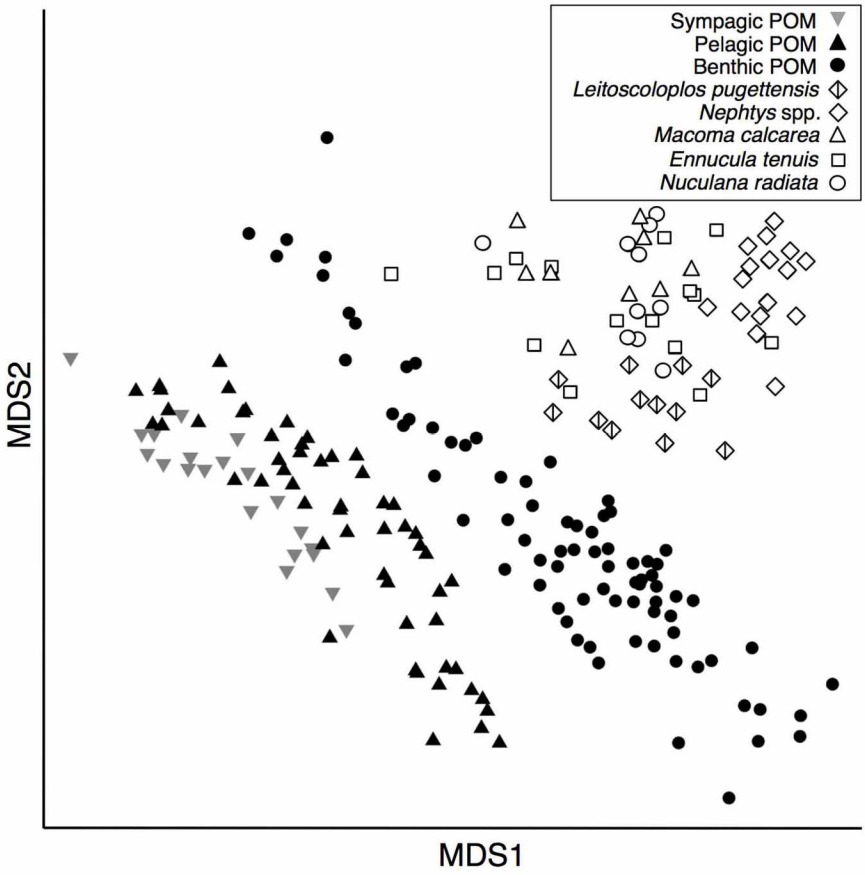

Figure 2.2 Non-metric multidimensional scaling plot of sympagic, pelagic, and benthic particulate organic matter and benthic invertebrate taxa. POM refers to particulate organic matter. Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, *N. radiata*. Distances are based on Bray-Curis similarity matrices using 71 fatty acids occurring in relative proportions > 0.1 %. 2D stress = 0.16.

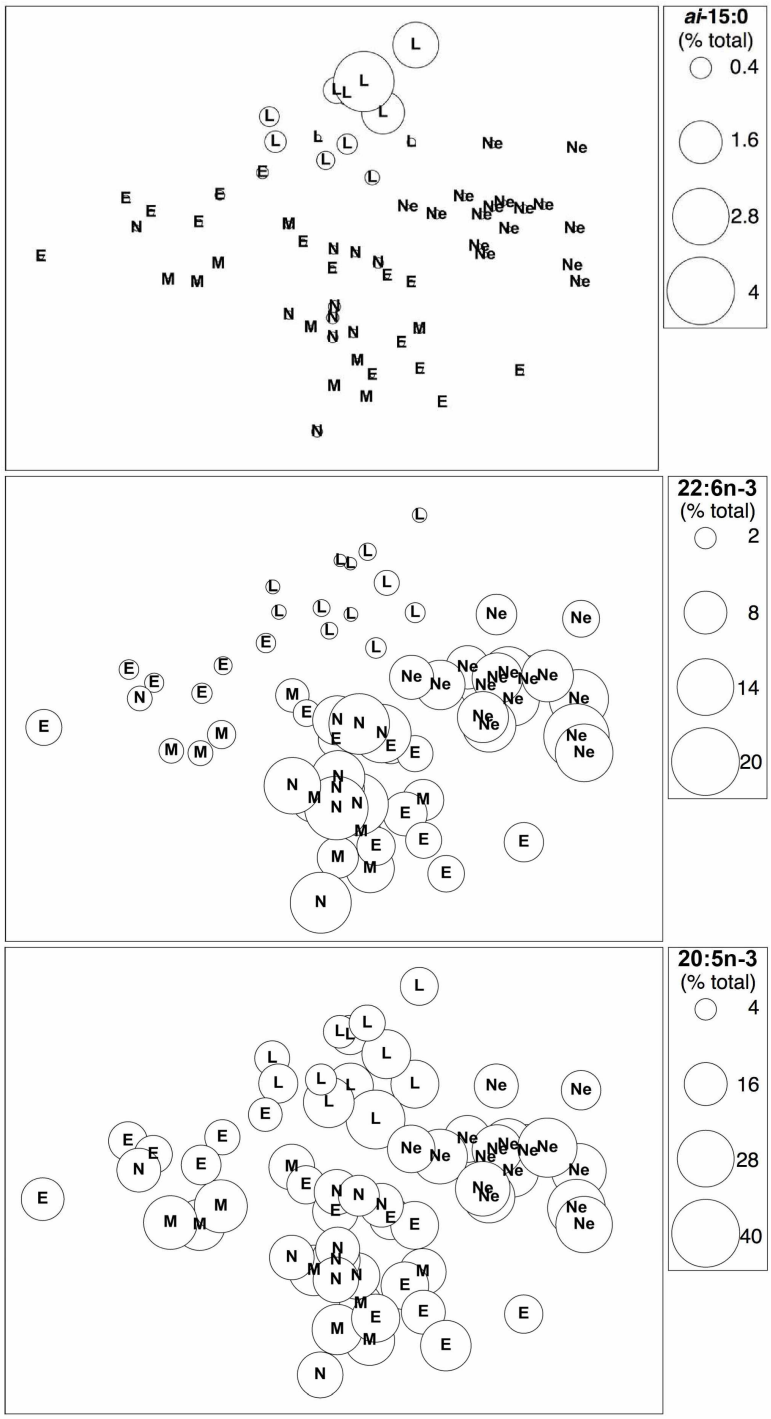

Figure 2.3 Non-metric multidimensional scaling plots showing the relative proportions of marker fatty acids from benthic invertebrate taxa. Bubble size corresponds to relative proportion (% total) of the bacterial marker fatty acid *ai*-15:0 (top), and algal marker fatty acids 22:6n-3 (middle) and 20:5n-3 (bottom). Polychaete taxa include *L. pugettensis* (L), and *Nephtys* spp. (Ne) and bivalve taxa include *M. calcarea* (M), *E. tenuis* (E), and *N. radiata* (N). Distances are based on Bray-Curis similarity matrices using 71 fatty acids occurring in relative proportions > 0.1 %. 2D stress = 0.19.

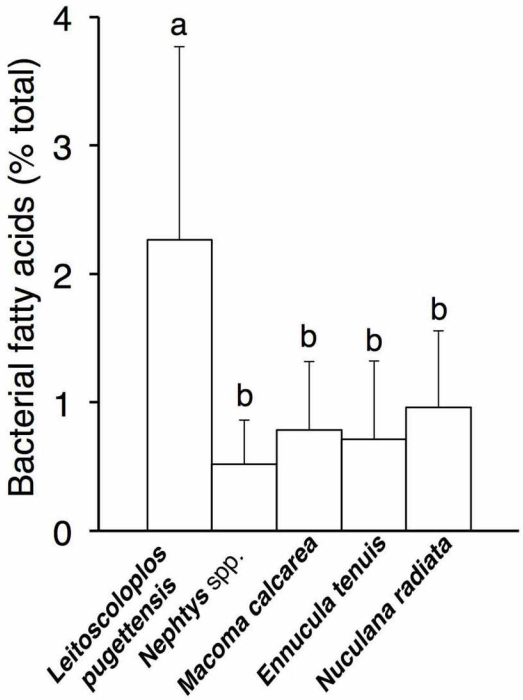

Figure 2.4 Relative proportions (% total) of the composite bacterial fatty acid marker in benthic invertebrate taxa. The composite bacterial marker is the sum of the fatty acids *i*-15:0, *ai*-15:0, *i*-17:0, *ai*-17:0 (% total, mean ± 1SD, individuals pooled by taxon). Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. Letters indicate statistically significant differences among taxa (Kruskal-Wallis one-factor ANOVA, $\chi^2 = 26$, df = 4, $p < 0.0001$, Mann-Whitney *U*-test with a Bonferroni adjustment for pairwise comparisons, $\alpha = 0.01$).

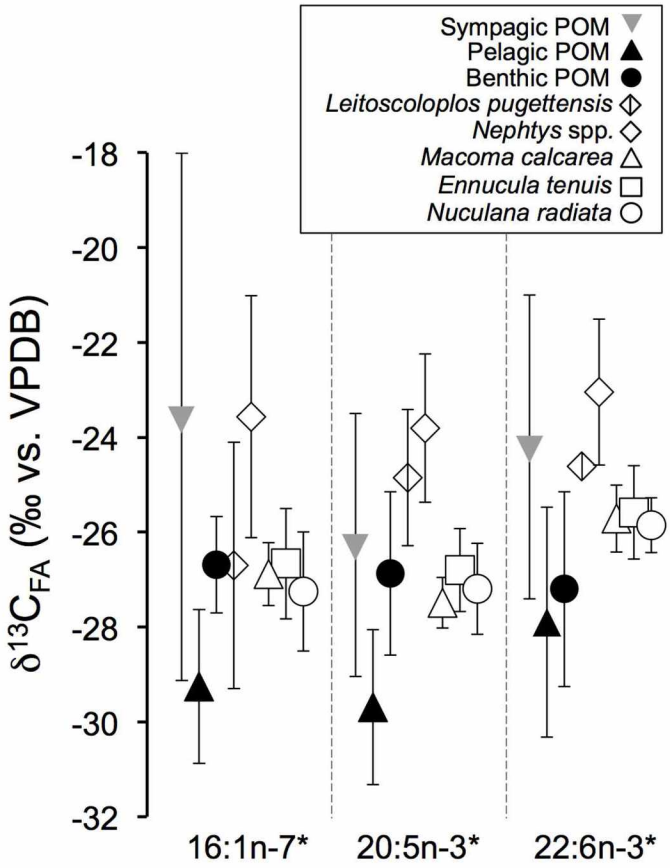

Figure 2.5 $\delta^{13}C$ values (‰) of algal marker fatty acids from sympagic, pelagic, and benthic particulate organic matter and benthic invertebrate taxa. Algal marker fatty acids are 16:1n-7, 20:5n-3, and 22:6n-3 (mean ± 1SD, individuals pooled by taxon). Sympagic and pelagic particulate organic matter (POM) values are taken from Wang et al. (2014) and Wang et al. (2015). Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. *indicates significant differences among taxa (POM excluded) (Kruskal-Wallis one-factor ANOVA, see Table 2.3 for Mann-Whitney *U*-test for pairwise comparisons, $\alpha = 0.01$).

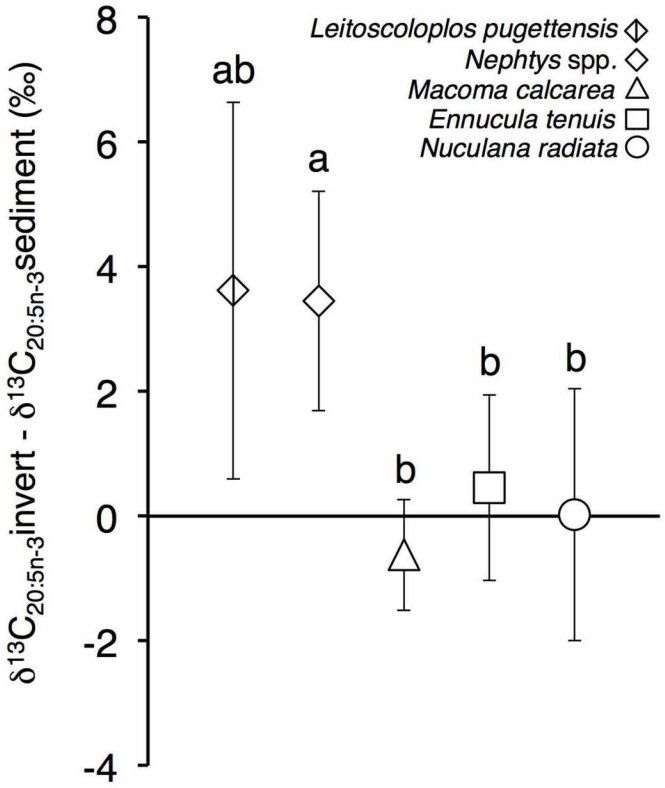

Figure 2.6 $\delta^{13}C$ values (‰) of the algal marker 20:5n-3 from benthic invertebrate taxa relative to those from surface sediments from the same location. Values are means ± 1SD, individuals pooled by taxon. Polychaete taxa include *L. pugettensis* (n = 6) and *Nephtys* spp. (n = 13) and bivalve taxa include *M. calcarea* (n = 9), *E. tenuis* (n = 13), and *N. radiata* (n = 8). Letters a-b indicate significant differences among taxa (Kruskal-Wallis one-factor ANOVA $\chi^2 = 25$, df = 4, $p < 0.0001$, Mann-Whitney *U*-test with a Bonferroni adjustment for pairwise comparisons, $\alpha = 0.01$).

Appendix 2.1 Site characteristics of stations where surface sediments were collected in the Bering Sea. Benthic invertebrate taxa were collected from stations (n = 82) when present. Shelf domain letters refer to the inner (I), middle (M), and outer (O) domains and the basin (Ba).

Date	Latitude (°N)	Longitude (°W)	Station no.	Depth (m)	Shelf Domain
3/25/2009	62.847	-169.022	36	36	M
3/23/2009	62.125	-169.250	24	40	I
3/27/2009	62.912	-174.069	41	72	M
3/18/2009	62.760	-173.681	9	68	M
3/15/2009	62.591	-174.756	3	70	M
3/18/2009	63.705	-172.540	8	50	M
3/13/2009	62.266	-175.193	1	80	M
3/19/2009	62.660	-171.200	11	44	M
3/16/2009	62.970	-173.388	5	71	M
3/23/2009	62.969	-169.156	21	38	M
4/10/2009	59.876	-175.215	19	120	O
5/4/2009	62.193	-175.140	98	80	M
4/14/2009	61.792	-176.802	29	113	O
4/8/2009	59.903	-170.394	10	62	M
4/15/2009	62.199	-175.145	32	80	M
4/24/2009	56.984	-170.290	66	72	M
4/7/2009	59.936	-170.000	9	56	M
5/6/2009	59.444	-174.082	115	115	O
4/5/2009	58.173	-169.100	3	69	M
4/16/2009	63.094	-173.291	35	62	M
4/29/2009	59.550	-175.096	85	133	O
5/1/2009	61.589	-173.709	92	72	M
4/20/2009	59.463	-167.787	50	35	I
4/22/2009	57.445	-169.754	58	66	M
4/26/2009	59.537	-175.205	69	133	O
4/18/2009	61.958	-167.991	45	28	I
4/4/2009	57.904	-169.317	1	72	M
4/28/2009	60.821	-174.384	83	90	M
6/20/2009	56.787	-167.874	32	101	M
7/1/2009	59.910	-169.803	98	98	M
7/5/2009	59.573	-175.250	122	136	O
7/7/2009	62.202	-173.118	140	62	M
7/3/2009	59.910	-178.206	114	114	O
6/16/2009	55.969	-163.143	10	82	I
6/22/2009	56.983	-170.288	49	71	M
7/2/2009	59.913	-175.206	108	108	O
6/22/2009	57.911	-169.248	45	70	M
7/6/2009	61.868	-169.876	133	47	M

Appendix 2.1 continued:

Date	Latitude (°N)	Longitude (°W)	Station no.	Depth (m)	Shelf Domain
3/13/2010	62.046	-175.067	1	83	M
3/14/2010	62.263	-175.402	2	84	M
3/14/2010	62.418	-174.696	3	74	M
3/15/2010	62.744	-173.751	6	69	M
3/16/2010	62.946	-173.461	8	71	M
3/18/2010	62.567	-172.187	15.2	53	M
3/20/2010	62.506	-171.764	23	50	M
3/21/2010	62.612	-170.166	26	36	M
3/23/2010	62.832	-171.420	37	50	M
3/24/2010	62.337	-173.932	39	66	M
3/26/2010	62.971	-173.941	41	79	M
3/27/2010	63.273	-173.735	43	78	M
3/28/2010	63.354	-173.221	47	72	M
5/11/2010	56.294	-171.052	2	141	O
5/13/2010	56.734	-179.910	7	104	Ba
5/15/2010	58.354	-171.803	24	102	M
5/17/2010	59.337	-175.611	39	138	O
5/19/2010	59.911	-178.953	49	489	Ba
5/23/2010	59.075	-170.174	66	67	M
5/25/2010	56.917	-167.317	71	78	M
5/27/2010	58.175	-169.907	81	72	M
5/29/2010	55.442	-168.068	87	203	O
5/30/2010	57.141	-163.809	94	67	M
6/2/2010	58.620	-170.286	124	72	M
6/3/2010	59.846	-171.838	134	74	M
6/4/2010	61.418	-173.736	147	74	M
6/5/2010	62.193	-175.155	156	79	M
6/5/2010	62.104	-175.291	158	61	M
6/8/2010	59.901	-175.202	170	119	O
6/10/2010	58.839	-168.165	179	46	I
6/10/2010	57.903	-169.247	184	67	M
6/23/2010	57.052	-167.449	33	70	M
6/24/2010	59.284	-167.618	39	39	I
6/25/2010	57.889	-169.223	45	67	M
6/27/2010	56.255	-171.110	53	189	Ba
6/27/2010	57.279	-173.841	60	191	Ba
6/29/2010	58.960	-173.873	69	123	O
7/1/2010	59.900	-169.202	84	47	I
7/2/2010	59.900	-175.200	94	118	O
7/4/2010	59.900	-178.200	99	142	O
7/7/2010	62.200	-169.849	124	41	M
7/7/2010	62.200	-173.116	132	60	M
7/8/2010	62.200	-175.905	139	90	M
7/9/2010	62.667	-173.383	145	68	M

Appendix 2.2 Lipid content (mg lipid/ mg wet weight) of benthic invertebrate taxa. Values are means ± 1SD, n = sample size, individuals pooled by taxon.

Taxon	n	% Lipid content
Leitoscoloplos pugettensis	6	13.6 ± 7.8
Nephtys spp.	8	2.3 ± 1.2
Macoma calcarea	4	1.7 ± 1.1
Ennucula tenuis	10	7.2 ± 8.9
Nuculana radiata	5	3.9 ± 2.2

Appendix 2.3 Relative proportions (% total) of fatty acids from sympagic, pelagic, and benthic particulate organic matter. Mean ± 1SD, n = sample sizes, sampleds pooled by particulate organic matter (POM) source. Sympagic POM = ice POM (i-POM), benthic POM = b-POM, pelagic POM = p-POM.

	i-POM	p-POM	b-POM
n	21	55	79
12:0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.7
13:0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 1.3
i-14:0	0.3 ± 0.3	0.3 ± 0.2	0.4 ± 0.3
14:0	6.9 ± 2.1	5.2 ± 1.9	5.5 ± 2.4
14:1n-9	0.0 ± 0.1	0.1 ± 0.2	0.3 ± 0.3
14:1n-7	0.2 ± 0.1	0.3 ± 0.3	0.3 ± 0.1
14:1n-5	0.6 ± 0.4	0.4 ± 0.2	0.4 ± 0.2
i-15:0	0.1 ± 0.1	0.3 ± 0.2	1.0 ± 0.9
ai-15:0	0.1 ± 0.1	0.2 ± 0.1	1.1 ± 0.8
15:0	0.4 ± 0.3	0.6 ± 0.2	1.0 ± 0.5
i-16:0	0.2 ± 0.3	0.4 ± 0.5	0.4 ± 0.3
16:0	12.0 ± 2.2	16.1 ± 4.2	17.0 ± 3.8
16:1n-11	2.1 ± 2.0	1.1 ± 0.9	0.3 ± 0.2
16:1n-9	0.4 ± 0.7	0.6 ± 0.8	1.4 ± 0.7
16:1n-7	15.7 ± 6.7	16.2 ± 12.9	27.8 ± 16.1
16:1n-5	0.3 ± 0.1	0.8 ± 0.7	0.9 ± 0.4
i-17:0	0.1 ± 0.4	0.2 ± 0.1	0.4 ± 0.4
ai-17:0	0.1 ± 0.1	0.4 ± 0.3	1.1 ± 1.7
16:2n-4	2.7 ± 0.9	1.1 ± 0.9	0.6 ± 0.3
17:0	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.2
16:3n-4	2.0 ± 1.5	1.1 ± 1.0	0.4 ± 0.3
17:1	0.1 ± 0.1	0.1 ± 0.1	0.6 ± 0.5
16:4n-1	8.2 ± 2.4	3.5 ± 4.0	0.8 ± 0.7
18:0	2.9 ± 2.1	6.8 ± 5.1	3.1 ± 1.9
18:1n-13	0.1 ± 0.3	0.2 ± 0.2	0.2 ± 0.2
18:1n-11	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
18:1n-9	8.9 ± 7.6	10.4 ± 10.5	4.3 ± 2.5
18:1n-7	0.5 ± 0.3	1.4 ± 0.7	4.3 ± 2.4
18:1n-5	0.2 ± 0.5	0.5 ± 0.8	0.8 ± 1.0
18:2d5,11	0.1 ± 0.0	0.2 ± 0.2	0.4 ± 0.2
18:2n-6	2.5 ± 1.3	3.3 ± 2.3	0.7 ± 0.3
18:2n-4	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2
18:3n-6	0.5 ± 0.4	0.2 ± 0.2	0.2 ± 1.0
18:3n-4	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.9
18:3n-3	0.3 ± 0.2	0.6 ± 0.6	0.4 ± 0.5
18:3n-1	0.2 ± 0.1	0.1 ± 0.1	0.8 ± 1.3
18:4n-3	2.4 ± 1.3	2.8 ± 1.8	0.6 ± 0.5
18:4n-1	0.2 ± 0.2	0.2 ± 0.3	1.1 ± 1.4
20:0	0.1 ± 0.1	0.2 ± 0.2	0.6 ± 0.4
20:1n-11	0.1 ± 0.1	0.1 ± 0.1	0.4 ± 0.5
20:1n-9	0.2 ± 0.2	0.6 ± 1.5	0.6 ± 0.5
20:1n-7	1.0 ± 1.3	1.5 ± 1.6	1.4 ± 1.0
20:4n-6	0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.5
20:5n-3	20.1 ± 6.8	11.7 ± 7.0	3.5 ± 2.4
22:0	0.2 ± 0.1	0.2 ± 0.2	0.5 ± 0.7
22:1n-11	0.1 ± 0.1	0.1 ± 0.1	1.8 ± 3.7
22:1n-9	0.7 ± 1.1	0.6 ± 0.5	2.2 ± 2.5

Appendix 2.3 continued:

	i-POM	p-POM	b-POM
22:1n-7	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
21:5n-3	0.2 ± 0.2	0.3 ± 0.2	0.3 ± 0.3
23:0	0.2 ± 0.2	0.4 ± 0.4	0.6 ± 0.4
22:5n-6	0.3 ± 0.1	0.3 ± 0.2	0.1 ± 0.1
22:4n-3	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4
24:0	0.2 ± 0.1	0.2 ± 0.2	1.7 ± 1.4
22:6n-3	2.3 ± 0.8	4.9 ± 3.2	1.0 ± 0.8
24:1	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 1.9

Appendix 2.4 Mean $\delta^{13}C$ values (‰) of non-marker fatty acids from benthic invertebrate samples. Values are means ± 1SD, n.d. = no data, individuals pooled by taxon. Polychaete taxa include *L. pugettensis*, and *Nephtys* spp. and bivalve taxa include *M. calcarea*, *E. tenuis*, and *N. radiata*. Letters a-d indicate significant differences among taxa (Kruskal-Wallis one-factor ANOVA, $p < 0.0001$, Mann-Whitney *U*-test with Bonferroni correction for pairwise comparisons, $\alpha = 0.01$).

	L. pugettensis	***Nephtys*** spp.	***M. calcarea***	***E. tenuis***	***N. radiata***
16:0	-24.7 ± 1.9^{ab}	-23.2 ± 2.2^{a}	-26.8 ± 0.9^{b}	-26.6 ± 0.9^{b}	-26.7 ± 1.4^{b}
18:0	-23.9 ± 2.2	-21.3 ± 1.4	-26.1 ± 1.0	-25.6 ± 1.0	-25.5 ± 1.0
18:1n-9	-23.1 ± 2.4	-22.2 ± 2.0	-24.6 ± 3.5	-24.8 ± 2.4	n.d.
18:1n-7	-23.5 ± 1.4^{ab}	-23.0 ± 1.3^{a}	-25.4 ± 0.9^{bc}	-26.9 ± 0.8^{c}	-26.6 ± 0.9^{c}
20:1n-7	-23.8 ± 1.4	-22.9 ± 2.9	-26.5 ± 2.9	-25.2 ± 0.9	-24.4 ± 2.2

Chapter 3 Resource partitioning between Pacific walruses and bearded seals during 2009-2011 in Alaska[1]

3.1 Abstract

Climate-mediated changes in Arctic sea ice phenology and primary production may alter seafloor food webs that sustain populations of Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). Interspecific resource competition could place an additional strain on ice-associated marine mammals already facing loss of sea ice habitat. Using fatty acid (FA) profiles, FA trophic markers, and FA stable carbon isotope analyses, we found that walruses and bearded seals partitioned food resources in 2009-2011. Interspecific differences in FA profiles were largely driven by variation in non-methylene FAs, markers of benthic invertebrate prey taxa, indicating varying consumption of specific benthic prey. We used Bayesian multi-source FA stable isotope mixing models to estimate proportional contributions of particulate organic matter (POM) from pelagic, benthic, and sympagic (ice algal) sources to these apex predators. Proportional contributions of FAs to walruses were dominated by pelagic (51 (32-73) %) and benthic (44 (17-67) %) POM sources. Bearded seals obtained a majority of FAs from benthic POM sources (62 (38-83) %) with considerable contributions from sympagic sources (27 (15-42) %). To interpret differences in the trophic pathways that sustained walruses and bearded seals, we compared the $\delta^{13}C$ values of algal FAs from walruses and bearded seals to those from benthic prey from different feeding groups from the Chukchi and Bering seas. Our findings suggest that 1) resource partitioning may mitigate interspecific competition, and 2) climate change impacts on Arctic food webs may elicit species-specific responses in these high trophic level consumers.

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3.2 Introduction

Extensive sea ice decline and a temporal shift in seasonal Arctic sea ice retreat have important implications for ice-associated marine mammals, such as Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). Both species primarily rely on benthic food resources (Lowry et al. 1980; Fay 1982; Fay et al. 1984) and use sea ice as diving platforms to access benthic feeding grounds in the Bering, Chukchi, and western Beaufort seas as the ice edge retreats northward each spring (Burns and Frost 1979; Fay 1982; Jay et al. 2012). Projected future changes in the sea ice environment may affect availability of and access to benthic prey, potentially increasing competition for dietary resources between walruses and bearded seals.

Sea ice conditions influence algal production within the sea ice, under the ice, and in open water, as well as deposition on the seafloor, thus affecting availability of benthic invertebrate populations (Grebmeier et al. 2006a; Bluhm and Gradinger 2008; Grebmeier 2012; Arrigo 2013; Boetius et al. 2013). In the Bering Sea, sea ice cover varies among years, fluctuating between "warm" and "cold" periods. In cold years (e.g., 2007-2014), sea ice melts in spring and releases sea ice algae (sympagic production), which sink to the benthos ungrazed, where they provide an important food subsidy to benthic fauna (Grebmeier et al. 2006a). Ice melt also releases nutrients needed to seed a phytoplankton bloom in the water column at the ice edge (pelagic production) (Sakshaug and Skjoldal 1989; Perrette et al. 2011). In contrast, warm years in the Bering Sea (e.g., 2001-2005) are characterized by earlier ice retreat (Stabeno et al. 2012), which may have implications for trophic pathways, including those that sustain benthic biomass. In the Bering Sea, earlier ice melt may result in intensified wind mixing, which prevents stratification and delays the development of pelagic blooms (Hunt et al. 2002, 2008, 2011). When a pelagic bloom occurs later in the season, pelagic algal grazers are abundant and consume the production before it sinks to the benthos (Walsh and McRoy 1986; Huntley and Lopez 1992). An ecological shift in which more production is partitioned to the pelagic realm and benthic prey resources concurrently decline (Grebmeier et al. 2006b; Grebmeier 2012) could result in increased competition among benthic-feeding marine mammals, such as walruses and bearded seals. However, recent observations of under-ice pelagic blooms in the adjacent Chukchi Sea suggest that export of production to the benthos may continue and possibly increase, given the potentially high biomass and large spatial extent of these early season blooms (Arrigo et al.

2012, 2014; Lowry et al. 2014). Additional research describing trophic linkages is necessary to anticipate shifts in algal production and deposition and any resulting changes in the benthic ecosystem in future climate scenarios.

Changes in sea ice availability, extent, and timing of retreat may impact access to benthic prey. Walruses and bearded seals utilize ice floes to passively access food resources on the Beringian shelf (Burns and Frost 1979; Ray et al. 2006). In recent years, however, ice retreat has occurred early and walruses have come ashore to terrestrial haul-out locations in large aggregations that have exceeded 30,000 animals (Jay and Fischbach 2008; Monson et al. 2013). Although walruses have used terrestrial haul-out sites on the Russian coast for several decades (Kryukova et al. 2014), the presence of large walrus herds on the northwest coast of Alaska has been less common, though not unprecedented (Collins 1940). This behavior may have consequences for foraging energetics and prey selection (Costa 1991; Rosen et al. 2007; Noren et al. 2012, 2014). At haul-out sites, marine mammals become central place foragers and risk rapid depletion of local benthic food resources (e.g., Costa 1991; Womble et al. 2009). As resources from benthic foraging grounds close to the terrestrial haul-out site become scarce, walruses may be forced to swim longer distances to access benthic hotspots (Jay et al. 2012; Ray et al. 2015), to occupy feeding grounds characterized by lower caloric density (Wilt et al. 2014), or to opportunistically consume prey they encounter in the pelagic realm (e.g., seabirds or seals - Collins 1940; Lowry and Fay 1984; Donaldson et al. 1995; Seymour et al. 2014a). Pelagic fishes make up a consistent, but small fraction of bearded seal diets (~7-11 % of stomach volume) (Lowry et al. 1980). The frequency of occurrence of certain forage fish species, including Arctic Cod (*Boreogadus saida*) and Walleye pollock (*Gadus chalcogrammus*), in bearded seal stomachs was greater in recent years (2003-2012) than during a historical period (1975-1984) (Crawford et al. 2015). This dietary shift did not, however, correspond to changes in ice cover (Crawford et al. 2015).

Bearded seals and walruses have coexisted for thousands of years on the Beringian shelf, sharing food resources and foraging grounds in the Bering and Chukchi seas (Repenning 1976; Lowry et al. 1980; Fay 1982; Harington 2008). Current understanding of ice-associated pinniped feeding ecologies is based on traditional ecological knowledge (TEK) (e.g., Noongwook et al. 2007; Huntington and Quakenbush 2013), stomach content analysis (e.g., Sheffield and Grebmeier 2009; Crawford et al. 2015), stable isotope analyses (e.g., Dehn et al. 2007; Seymour

et al. 2014a, 2014b) and, occasionally, direct observations of foraging (e.g., Donaldson et al. 1995; Lovvorn et al. 2010). Fatty acids (FAs) have been used to infer dietary niche separation between walruses and bearded seals (Budge et al. 2007) and to examine possible competition over benthic resources (Cooper et al. 2009; Wang et al. 2015b). More recently, compound-specific stable isotope analysis (CSIA) of FAs has been used as a tool to estimate the proportional contribution of algal FA sources to ice seals (Wang et al. 2016), as well as other consumers in Arctic and sub-Arctic marine food webs to describe trophic connectivity (Sun et al. 2004, 2007; Budge et al. 2008; Graham et al. 2014; Wang et al. 2015a; Oxtoby et al. in revision). In this study, we used FAs (trophic markers, profiles, and CSIA of FAs) to examine differences between the diets of walruses and bearded seals during 2009-2011, in order to contrast niche partitioning during a set of "cold" years with those observed during "warm" years (Budge et al. 2007; Cooper et al. 2009). Our main objectives were to: 1) describe the degree of dietary overlap between walruses and bearded seals, 2) estimate the proportional contributions of benthic, sympagic, and pelagic production sources to each species, and 3) interpret differences in diet and trophic pathways between walruses and bearded seals by relating the stable carbon isotope composition of their algal FAs to those from benthic invertebrate prey from distinct feeding groups in the Chukchi Sea (this study) and the Bering Sea (Oxtoby et al. in revision).

3.3 Material and methods

3.3.1 Sample acquisition

Walrus specimens were opportunistically sampled during spring/summer subsistence harvests in 2009 (n = 4), 2010 (n = 44), and 2011 (n = 9) near the communities of Gambell and Savoonga on St. Lawrence Island, Alaska (Fig. 3.1, Table 3.1). Alaskan Native subsistence hunters provided samples for scientific research in collaboration with the U.S. Fish and Wildlife Service (USFWS), U.S. Geological Survey, the Eskimo Walrus Commission, and the North Slope Borough Department of Wildlife Management. Walrus samples were collected under the authority of permit number 50 CFR 18.23(a)(3)(b)(1) and held at University of Alaska Fairbanks (UAF) under a Letter of Authorization from USFWS to L. Horstmann. Bearded seals were collected in 2009 (n = 10) and 2010 (n = 20) in cooperation with Alaskan Native subsistence hunters from the communities of Savoonga, Little Diomede, and Point Hope (Fig. 3.1, Table 3.1) and the Alaska Department of Fish and Game (ADF&G) Arctic Marine Mammal Program as

part of a long-term biomonitoring program (National Marine Fisheries Service Scientific Research Permit No. 358-1787). Additional information concerning bearded seal specimens is described in Wang et al. (2016).

Subsistence hunters recorded information about individual animals harvested, including sex, stomach contents, lactation status, presence of a calf, and body condition of the animals. Estimated ages of select walruses and bearded seals were obtained by sectioning teeth to determine the number of growth layer groups in cementum, which correspond to the age of the animal (Mansfield and Fisher 1960) (Matson's Laboratory, Montana, USA). All animals included in this study were adults (> 4-5 years of age) based on tooth age estimates or hunters' evaluations of morphological and reproductive characteristics of the animals. Blubber samples from muscle to skin were taken from the trunk of the body immediately after death. Due to air temperatures below freezing, samples froze on site and were shipped frozen to UAF, where they were immediately wrapped in aluminum foil, and sealed in plastic bags for storage at -80°C.

Benthic invertebrate specimens (n = 160 from seven taxa) were collected at 14 stations on the Chukchi Sea shelf in August and September 2012 during the Russian-American Long-term Census of the Arctic (RUSALCA), the Chukchi Sea Offshore Monitoring In Drilling Area (COMIDA), and the Arctic Ecosystem Integrated Survey (Arctic EIS) research expeditions. Samples were collected using bottom trawls or van Veen grabs at depths ranging from 34-58 m. Benthic prey consisted of an omnivore (the snow crab *Chionoecetes opilio)*, a subsurface deposit-feeder (the bivalve *Nuculana radiata*), and suspension/surface deposit-feeders (the bivalves *Liocyma fluctuosa*, *Serripes groenlandica*, *Astarte* spp., *Macoma* spp., *Ennucula tenuis*). Invertebrate samples were frozen at -20°C, then freeze-dried in a Virtis Freeze Dryer (model 52; The Virtis Company, NY, USA) while on board the ship. Particulate organic matter (POM) samples were collected in collaboration with the Bering Sea Ecosystem Study (BEST-BSIERP) in 2009 and 2010. Samples were collected during the months of March-July of each year from varying locations across the Bering Sea shelf. POM was sampled from ice cores (i-POM), from pelagic phytoplankton production (p-POM) (additional details are described in Wang et al. 2014), and surface sediment scrapes (b-POM). Foraging grounds for walruses and bearded seals span the Bering and Chukchi seas (e.g., Lowry et al. 1980; Fay 1982; Jay et al.

2012); therefore, inclusion of invertebrate specimens and POM samples from both regions was appropriate.

3.3.2 Sample preparation and lipid extraction

In preparation for lipid extraction, blubber samples were placed on a sterile glass cutting board, and a sterile knife was used to trim the outermost layer of blubber away to expose inner blubber layers. A longitudinal sample from the exposed muscle to the skin layer (full blubber depth) was removed and weighed (~1 g). Wet weights and dry weights were taken for invertebrate specimens (Mettler 200 analytical balance Greifensee, Switzerland). Invertebrate samples were then homogenized and stored in crimp top vials at -80°C prior to lipid extraction. To prepare the sediment samples, all visible macroinfaunal material was removed from sediment samples. Samples ranging from 3 to 25 g of freeze-dried sediment were weighed out. Details regarding i-POM and p-POM sample preparation and lipid extraction are described in Wang et al. (2014).

Lipids from full-depth blubber and from sediment samples were solvent extracted using a modified Folch procedure in a ratio of 8:4:3 of chloroform, methanol and deionized water (Folch 1957; Budge et al. 2006). Invertebrate samples were collected and analyzed as part of a separate research initiative, so the methods for their extraction differed slightly. Invertebrate specimens were lipid extracted using an accelerated solvent extraction (ASE) system (Dionex ASE 200, CA, USA). Approximately 0.5 g hydromatrix (Dionex, CA, USA) was combined with a subsample of 0.5 g homogenized freeze-dried tissue sample. An 11 ml stainless steel thimble was assembled with two cellulose filters and a thin layer of sand before the hydromatrix and tissue mixture was added. An additional cellulose filter was placed at the top before it was loaded into an ASE system. Dichloromethane (DCM, Fisher Thermo-scientific, Fair Lawn, NJ, USA) with butylated hydroxytoluene (BHT; Sigma Chemical, St. Louis, MO, USA) was added at 100 mg/L to prevent lipid oxidation. The extraction occurred at 85°C under 1500 psi nitrogen with two static cycles of 5 min each. Fatty acid methyl esters (FAME) for all samples were prepared from lipid extracts using an acidic transesterification procedure according to Budge et al. (2006).

3.3.3 Sample analysis

Relative proportions of individual FAs from blubber and sediment samples were measured using a Perkin Elmer Autosystem II gas chromatograph (GC) (Perkin Elmer, Boston,

MA, USA) with a flame ionization detector (FID) containing a 30 m 0.25 mm i.d. column coated with 50 % cyanopropyl polysiloxane (0.25 mm film thickness; J&W DB-23; Folsom, CA, USA). Samples (1 μL of FAME in hexane) were injected in splitless mode and analyzed according to a temperature program detailed in Budge et al. (2006). Each sample was analyzed in duplicate and fatty acid proportions were averaged. FA identities were determined by cross referencing retention times with those from an in house standard (menhaden oil) containing FAs previously identified using GC mass spectrometry (MS) (Thermo Finnigan Polaris Q; Bremen, Germany).

FAs from invertebrate samples were analyzed for their relative concentrations by adding an internal standard (23:0 at 1 mg/20 mg lipid) prior to methylation. Esterified lipid samples were dried (TurboVap) and FAMEs re-suspended in hexane to 20 mg/ml. The FAs 16:1n-7 and 20:5n-3 were identified by comparing peak retention times in gas chromatography (GC-FID, model 6850, Agilent Technologies, Wilmington, DE) to a known FA standard (Supelco, 189-19). The Supelco 189-19 standard was used to create a calibration curve from 0.1 to 1.0 mg for quantification of 16:1n-7 and 20:5n-3. Calibration curves for both FAs were extended utilizing FAME standards (Sigma Aldrich, Saint Louis, MO, USA) to reach concentrations of 5 mg/ml to encompass the concentration range found in samples. FA areas were corrected using Ackman response factors (Ackman and Sipos 1964) because they vary slightly for different FAs due to the interaction of FAs with the GC flame ionization detector (FID). The Ackman response factor is the recorded areas of the calibration curve divided by the 18:0 area values. The FA 18:0 was used as a baseline reference for Ackman response factors because it elutes in the middle of the run for most marine samples (Ackman and Sipos 1964). Corrected FA areas were then related back to the area of the internal standard.

$\delta^{13}C$ values of individual FAME from all samples (walrus, bearded seal, sediment, and invertebrate tissues) were analyzed at the Alaska Stable Isotope Facility (ASIF) using a GC (Thermo Scientific Trace GC Ultra) linked to an isotope ratio mass spectrometer (IRMS - Thermo Finnigan Delta V) through a combustion interface (IsoLink; www.isolink.com). The GC column, temperature program, and mode of injection were the same as for the GC-FID analyses used for blubber and sediment samples. 1 μL of FAME in hexane was injected at a sample concentration of FAME adjusted to generate a voltage of 500 - 3000 mV for 20:5n-3. We used a FAME standard consisting of 16:0 and 18:0 (Nu-Chek Prep, Inc.; Elysian MN), which we

injected throughout sample runs to track analytical error (n = 20 injections), which was < 0.1 ‰ and < 0.2 ‰, respectively (expressed as 1 SD of 16:0 and 18:0).

Stable carbon isotope ratios of FAs in a sample are described using conventional delta (δ) notation in parts per thousand (‰) and are expressed as follows: $\delta^{13}C = [(^{13}C/^{12}C_{standard}/^{13}C/^{12}C_{sample})-1]*1000$, where the standard is the international reference material Vienna Pee Dee Belemnite (VPDB). We analyzed a standard mixture containing eight calibrated *n*-alkanoic acid esters (Mixture F8, Indiana University Stable Isotope Reference Materials), where r^2 of the known *versus* expected correlation was > 0.99, to calibrate $\delta^{13}C$ values of individual FAs. We also corrected $\delta^{13}C_{FA}$ values to account for carbon added during transesterification using the following equation (Eq. 3.1):

$$(3.1) \quad \delta^{13}C_{FA} = [(n+1)(\delta^{13}C_{FAME})-(\delta^{13}C_{methanol})]/n$$

where $\delta^{13}C_{FA}$ is the adjusted value of the FA of interest, n is the number of its carbon atoms, $\delta^{13}C_{FAME}$ is the calibrated value of the FAME, and $\delta^{13}C_{methanol}$ is the stable isotope composition of the carbon contributed by the methanol (Abrajano et al. 1994). $\delta^{13}C_{methanol}$ ($\delta^{13}C_{methanol}$ = -49 ‰) was calculated by subtracting the $\delta^{13}C$ value of esterified 16:0 and 18:0 standards from the corresponding $\delta^{13}C$ values of their free FAs (Wang et al. 2014, 2015a).

FAs are expressed using the nomenclature A:Bn-X, where A indicates the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group of a FA. Non-methylene interrupted (NMI) dienoic and trienoic FAs are distinguished from methylene interrupted FAs by the symbol Δ (e.g., 20:2Δ5,11) and double bond positions are given relative to the carboxylic acid functionality. *Iso*- and *anteiso*-methyl branched FAs are further identified by lowercase italicized letters (e.g., *i*-15:0, a FA with 15 carbon atoms, 0 double bonds and a methyl branch on the second to last carbon atom in the chain). FA data (77 individual FAs) for bearded seals and walruses are provided in Appendix 3.1.

We report relative proportions and summed relative proportions of FAs considered to be markers of specific sources (reviewed in Parrish 2013). NMI FAs, which are synthesized exclusively by benthic invertebrate taxa (Paradis and Ackman 1977; Joseph 1982; Kawashima

2005; Barnathan 2009; Monroig et al. 2012), were used as "benthic" markers (Table 3.2). The sum of *iso*- and *anteiso*-methyl branched FAs with an odd number of carbon atoms (*ai*-15:0, *i*-15:0, *ai*-17:0, *i*-17:0) was used as a marker of bacteria (Volkman et al. 1980; Budge and Parrish 1998) (Table 3.2). The FAs 16:1n-7, 20:5n-3, and 22:6n-3 are considered to be algal in origin (e.g., reviewed in Dalsgaard et al. 2003), so they were used as isotopic end members for compound-specific stable isotope multi-source mixing models. Models describing proportional contributions of POM used $\delta^{13}C$ values from 16:1n-7, 20:5n-3, and 22:6n-3, whereas those describing contributions from invertebrate feeding groups only included 16:1n-7 and 20:5n-3 because 22:6n-3 was not measured in invertebrate specimens.

3.3.4 Data analyses

Multivariate non-parametric procedures were performed to describe differences in FA profiles and NMI FA profiles based on 77 FAs that were present in proportions > 0.1 % of the total between species and by sex within species. FA percentage data were transformed using a log(X+1) transformation prior to statistical analysis. We measured differences in profiles using a two-factor nested permutational multivariate analysis of variance (PERMANOVA) with species and sex (nested) as factors. Similarity percentage routines (SIMPER) were employed to identify the FAs that contributed most to dissimilarities in the FA and NMI FA profiles (Fig. 3.2). PERMANOVA and SIMPER routines were performed in PRIMER (version 6, Primer-E Ltd). Univariate data met assumptions for parametric analysis, so a two-factor nested ANOVA was used to compare the relative proportions of individual NMI FAs (Fig. 3.3), the sum of NMI FAs, a composite bacterial marker (sum of *ai*-15:0, *i*-15:0, *ai*-17:0, and *i*-17:0) (Table 3.2), and $\delta^{13}C$ values of algal FAs (16:1n-7, 20:5n-3, and 22:6n-3) (Fig. 3.4) between species and by sex (nested) within species. Tukey Honest Significant Differences test was performed for pairwise comparisons at a 95 % significance level ($\alpha = 0.05$) (R version 3.2.2).

We used a Bayesian mixing model (SIAR, R version 3.2.3) (Parnell et al. 2010) to estimate the proportional contributions of FAs from POM sources (i-POM, p-POM, and b-POM) to walruses and bearded seals. Models were based on $\delta^{13}C$ values of the algal FA markers 16:1n-7, 20:5n-3, and 22:6n-3, so we ran four models using varying combinations of $\delta^{13}C$ values (Table 3.3) and run with and without concentration dependencies (Appendix 3.2) for comparison (as in Wang et al. 2016). We assumed a FA trophic enrichment factor of 0 (Budge et al. 2011; Wang et

al. 2016). Male and female walruses were analyzed separately, whereas sexes were combined for bearded seals, because there were no significant differences in FA sources for bearded seals between sexes (see Results 3.4.1). Results are presented as means (95 % credibility interval) (Bayesian confidence interval).

We compared $\delta^{13}C$ values of algal FAs from walruses and bearded seals to those from benthic prey from distinct feeding groups from the Chukchi Sea (an omnivore, a subsurface deposit-feeder, and suspension/surface deposit-feeders) (this study) and the Bering Sea (a predator, a head down deposit-feeder, and suspension/surface and subsurface deposit-feeders) (Oxtoby et al. in revision) (Fig. 3.5). Benthic invertebrate specimens from the Chukchi Sea were included in this study to extend the geographic coverage of benthic prey from the Bering Sea (Oxtoby et al. in revision) to known summer feeding grounds for Pacific walruses (e.g., Ray et al. 2006). Although benthic prey from the Chukchi Sea were collected during a different year (2012) than the walruses and bearded seals (2009-2010), mean $\delta^{13}C$ values of algal FAs (16:1n-7, and 20:5n-3) from *M. calcarea*, *E. tenuis*, and *N. radiata* collected in 2009-2010 from the Bering Sea (Oxtoby et al. in revision) were very similar to those reported here (differences between mean values ranged from 0.4-0.7 ‰ and 0.7-1.3 ‰ for 16:1n-7 and 20:5n-3, respectively) (Fig. 3.5). This supported inclusion of specimens from 2012 given the lack of interannual and geographic variability.

We chose not to include a mixing model to estimate proportional contributions of benthic prey groups to walrus and bearded seal diets because walruses and bearded seals consume a wide variety of prey taxa, including pelagic prey (Dehn et al. 2007; Sheffield and Grebmeier 2009; Huntington and Quakenbush 2013; Crawford et al. 2015). As a result, model estimates of the proportional contributions of benthic prey would not be a comprehensive representation of the entire diets of these two species. Instead, we used $\delta^{13}C$ values of algal FAs from benthic invertebrate taxa that are examples of varying feeding types to qualitatively interpret differences in their diets (Fig. 3.5).

3.4 Results

3.4.1 Fatty acid profiles and markers

FA profiles of walruses and bearded seals were significantly different (PERMANOVA, $P < 0.01$) (Fig. 3.2). Within species, FA profiles differed between male and female walruses (PERMANOVA, $P = 0.03$), but not between male and female bearded seals (PERMANOVA, $P = 0.07$) (Fig. 3.2). Walruses that were multivariate outliers based on an nMDS biplot (Fig. 2) were males ($n = 2$) harvested in Savoonga in 2009 and 2010. According to hunters' observations, one specimen was severely emaciated with an empty stomach; the other was healthy. FAs that contributed most to differences between walruses and bearded seals in FA profiles were the NMI FAs 20:2Δ5,11, 22:2Δ7,15, and 24:1, 16:3n-3, 20:2n-9, 23:0, and *ai*-15:0 (SIMPER) (Fig. 3.2). Additional NMI FAs (22:2Δ7,13, 20:2Δ5,13, 20:3Δ5,11,14) were among the FAs that collectively contributed up to 45 % of the variation in FA profiles between species (Fig. 3.2).

NMI FA profiles of walruses differed significantly from those of bearded seals (PERMANOVA, $P < 0.01$). Walruses had significantly higher relative proportions of select NMI FAs (20:2Δ5,11, 20:2Δ5,13, 20:3Δ5,11,14, 22:2NMID, 22:2Δ7,13) than bearded seals (Two-factor nested ANOVA, $P < 0.01$) with the exception of 22:2Δ7,15, which was greater in bearded seals (Two-factor nested ANOVA, $P < 0.01$) (Table 3.2, Fig. 3.3). Due to large relative proportions of 22:2Δ7,15 in bearded seals, the sum of the relative proportions of NMI FAs did not differ between species (Two-factor nested ANOVA, $P = 0.10$) (Table 3.2). Within species, NMI FA profiles did not vary significantly between sexes (PERMANOVA, $P = 0.12$). However, when multivariate outliers were removed from the dataset (Fig. 3.2b, $n = 2$), sex-specific differences in NMI FA profiles were detected among walruses (PERMANOVA, $P < 0.01$); bearded seals were unaffected (PERMANOVA, $P = 0.21$). No significant differences were detected between sexes for either species for five of the six NMI FAs (Two-factor nested ANOVA, $P > 0.10$). Differences in the relative proportion of 22:2NMID (non-methylene interrupted dienoic) fatty acid between male and female walruses, however, were significant (Tukey Honest Significant Differences test, $P < 0.01$).

The relative proportion of a composite bacterial FA marker was significantly higher in walruses relative to bearded seals (Two-factor nested ANOVA, $P < 0.01$) (Table 3.2); no

differences between sexes were detected for either species (Two-factor nested ANOVA, P = 0.90).

3.4.2 Stable carbon isotope analysis of fatty acids

$\delta^{13}C$ values of algal marker FAs (16:1n-7, 20:5n-3, and 22:6n-3) were higher in bearded seals relative to walruses (Two-factor nested ANOVA, $P < 0.01$), but did not vary between sexes in either species (Two-factor nested ANOVA, $P > 0.10$) (Fig. 3.4). Mean $\delta^{13}C$ values of algal FAs ranged from -28.9 ± 1.2 ‰ (20:5n-3) to -26.6 ± 1.1 ‰ (22:6n-3) in walruses (mean ± 1SD, n = 41, sexes pooled) and from -26.5 ± 0.8 ‰ (20:5n-3) to -24.1 ± 0.8 ‰ (16:1n-7) in bearded seals (mean ± 1SD, n = 28, sexes pooled).

We present a range of estimates generated from Bayesian multi-source FA stable isotope mixing models that incorporated various combinations of algal marker FAs and their concentration dependencies (Tables 3.3, 3.4; see Appendices 3.2, 3.3 for additional information about sources). The model containing 20:5n-3 and 22:6n-3 provided the most reliable estimates due to greater sample sizes compared with models containing 16:1n-7, which was not always measurable due to GC coelution of monounsaturated and saturated FAs containing 16 carbon atoms. Through their diets, walruses and bearded seals indirectly obtained substantial contributions of FAs from b-POM (ranging from 44 (17-67) % in walruses to 62 (38-83) % in bearded seals) (Table 3.3). Bearded seal diets contained higher indirect contributions of i-POM (27 (15-42) %) compared with walruses (13 (2-23) %) (Table 3.3). In contrast, bearded seal diets contained lower indirect contributions from p-POM (10 (1-22) %) relative to walruses (51 (32-73 %) (Table 3.3). Proportional indirect contributions of POM sources were similar between male and female walruses (Table 3.3). However, Bayesian credibility intervals were larger for walruses relative to bearded seals for POM sources.

The stable carbon isotope composition of algal FAs (16:1n-7 and 20:5n-3) from male and female walruses was similar to algal FAs from suspension/surface deposit-feeding bivalves and from an example of a subsurface deposit-feeder (the bivalve *N. radiata*) from the Chukchi and Bering seas (Fig. 3.5). In contrast, $\delta^{13}C$ values of algal FAs from male and female bearded seals clustered more closely to *C. opilio*, an epibenthic omnivore, and to *Nephtys* spp, a predatory polychaete (Fig. 3.5).

3.5 Discussion

Walruses and bearded seals collected from 2009-2011 had distinct diets consistent with earlier studies (Budge et al. 2007; Cooper et al. 2009). FA analyses revealed that interspecific dietary differences were revealed by variation in benthic prey taxa, as evidenced by differences in individual benthic FA markers. However, the sum of all benthic marker FAs did not significantly differ between walruses and bearded seals, indicating a similar general reliance on benthic food resources. Benthic and pelagic POM sources contributed the majority of FAs to the prey taxa that sustained walruses, whereas benthic and, to a lesser extent, sympagic POM sources contributed FAs to the prey resources that sustained bearded seals. We posit that differences in the diets and trophic pathways that sustain walruses and bearded seals resulted from higher predation on surface and subsurface deposit-feeding bivalves by walruses and from higher predation on predatory and epibenthic omnivorous prey by bearded seals. Benthic marker FA data from this study mirror those reported from 2002 (Budge et al. 2007; Cooper et al. 2009), suggesting that resource partitioning between species has not changed over time despite interannual variability documented in bearded seal diets based on their benthic marker FAs (Cooper et al. 2009; Wang et al. 2015b).

3.5.1 Interspecific and intraspecific variation in diet

Walruses are gregarious animals that travel in large herds and occasionally congregate en masse at terrestrial haul-out sites (Fay 1982; Jay and Fischbach 2008), whereas bearded seals tend to be solitary (Cleator et al. 1989; Simpkins et al. 2003). Increased foraging pressure on localized food resources by walrus herds could oblige individual walruses to consume a greater diversity of available prey. In contrast, bearded seals, as solitary individuals, might have a greater ability to forage selectively on preferred food resources, assuming that prey are locally available. High variation in the stable isotope composition of algal FAs from walruses suggests that individual walruses consistently targeted specific food resources within the broad range of prey utilized by the group or population. In contrast, bearded seals exhibited lower dietary breadth and greater consistency in prey selection among individuals relative to walruses. More than 100 prey taxa have been identified in the stomach contents of Pacific walruses (Sheffield and Grebmeier 2009), whereas bearded seals have been shown to rely heavily on crustacean prey and are not known to consume seabirds and seals (Lowry et al. 1980; Crawford et al. 2015),

observations that may also explain greater variation in the diets of walruses compared to bearded seals.

Variation in the proportional contributions of algal sources to their prey also differentiated the diets of these predators. Higher ice algal contributions to bearded seals likely reflect greater consumption of prey taxa such as *Nephtys* spp. (a predatory polychaete). *Nephtys* spp. from the northern Bering Sea were isotopically similar to bearded seals, an observation corroborated by previous research that established that they are a major prey taxon in bearded seal diets (Lowry et al. 1980). *Nephtys* spp. were also characterized by high δ^{13}C-FA values that were attributed to indirect consumption of i-POM through their prey (Oxtoby et al. in revision). Bearded seals were also isotopically similar to *C. opilio* (snow crab), an example of an omnivore species in this study. *C. opilio* consumes a broad range of benthic taxa, including bivalves, gastropods, polychaetes, amphipods, and other crustaceans (Kolts et al. 2013a, 2013b, Divine et al. 2015). Carbon isotope ratios of snow crabs (total organic carbon - TOC) were high compared with bivalves (Kolts et al. 2013b), similar to our compound specific results. However, whether these values reflect an ice algal or benthic signature is unclear. Finally, an ice algal signature could be transferred to bearded seals via Arctic Cod, which consumes ice-associated amphipods (Lowry and Frost 1981; Lønne and Gulliksen 1989).

Forage fishes (e.g., Arctic Cod, Canadian eelpout- *Lycodes polaris*, and Longear eelpout- *Lycodes seminudus*) are an important food resource for bearded seals (Lowry et al. 1980; Crawford et al. 2015). However, there were no available datasets of CSIA-FA from adult forage fishes to elucidate dietary differences that may have resulted from consumption of pelagic prey. These forage fish species typically have a ratio of 20:1n-9 to 22:1n-11 greater than 1 (Iverson et al. 2002; Falk-Petersen et al. 2004, Dissen 2015), which also was characteristic of bearded seals included in this study. Consequently, it is likely that these, and possibly other, fish species may account for part of the dietary signature in bearded seals. Compound-specific stable isotope analysis of additional prey taxa could offer further insight into the relative importance of prey from different feeding groups to the diets of walruses and bearded seals; for example, shrimp, polychaetes, fish, and bivalve species such as *Mya* spp. that are dominant prey items in bearded seals and walrus stomachs (Lowry et al. 1980; Dehn et al. 2007; Sheffield and Grebmeier 2009).

Proportional contributions of i-POM to bearded seals are lower than recent estimates that also used FA stable isotope mixing models to apportion POM sources to bearded seals (Wang et al. 2016). Our model included a benthic source (b-POM), which is characterized by $\delta^{13}C$ values for 20:5n-3 comparable to those of i-POM, possibly due to isotopic fractionation associated with microbial degradation of algal material (see Sun et al. 2004; Oxtoby et al. in revision for further discussion of isotopic fractionation of b-POM). A model containing only two sources (i-POM and p-POM) would apportion any contributions from b-POM to i-POM. Indeed, the sum of i-POM and b-POM contributions from our model was roughly equal to the contribution estimated from i-POM alone to bearded seal diets in 2009 and 2010 (Wang et al. 2016).

Walruses consumed prey that relied primarily on benthic and pelagic carbon sources, with only a small subsidy from ice algal carbon. We attribute this to greater consumption of suspension/surface and subsurface deposit-feeding bivalves, which have been shown to consume the organic matter available in surface sediments, irrespective of its origin (Oxtoby et al. in revision). The strong pelagic signal in primary consumers, such as suspension/surface deposit-feeders and subsurface deposit-feeders in the benthic environment, likely results from the dominance of pelagic production to total annual primary production in the Arctic and sub-Arctic marine ecosystem (McRoy and Goering 1974; Gosselin et al. 1997).

There were differences in some FA dietary proxies between male and female walruses, suggesting sex-specific differences in diet, whereas there were no differences in FA dietary proxies between male and female bearded seals. Sexual segregation in summer likely explains dietary differences between male and female walruses (Jay and Hills 2005; Ray et al. 2006). In summer, females and calves migrate northward to the Chukchi Sea while males migrate south to Bristol Bay and locations along the Russian coast including the Gulf of Anadyr to forage (Fay 1982, Ray et al. 2006). We posit that sex-specific differences in FA profiles can be attributed to incorporation of prey FAs during sexual segregation in the previous summer. Male walruses rarely feed during the winter reproductive period when females are present, so their blubber may preserve a summer foraging signal (Ray et al. 2006). Differences in diet could also result from the ability of male walruses to dive deeper while foraging and to obtain "atypical" prey (e.g., ringed (*Pusa hispida*) and bearded seals) (Lowry and Fay 1984; Huntington and Quakenbush 2013, Seymour et al. 2014b). Dietary differences could also arise from selective foraging by

reproductive females for high lipid content prey to support high energetic demands (Noren et al. 2012, 2014).

3.6 Conclusions

Walruses consumed prey from a distinct and slightly broader trophic spectrum compared with bearded seals in the recent study years. Both species had similar proportions of benthic prey markers, supporting the idea that they are both benthic foragers. However, differences in individual benthic markers indicated that they relied on different benthic prey. Walruses and bearded seals were also sustained by two distinct trophic pathways characterized by different contributions of algal organic matter sources to their respective prey; specifically, higher predation on surface and subsurface deposit-feeding bivalves by walruses and higher predation on predatory and epibenthic omnivorous prey by bearded seals. Resource partitioning of benthic invertebrate prey may facilitate the co-occurrence of these two species. Given that the dominant trophic pathways supporting each consumer are distinct, climate-induced changes in algal production in the Arctic could affect walruses and bearded seals differently.

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3.9 Tables

Table 3.1 Sample sizes of Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*) analyzed for fatty acid composition and stable carbon isotope values ($\delta^{13}C_{FA}$) of fatty acids. (M = males, F = females, U = unknown sex). See Figure 3.1 for harvest locations.

	Pacific walrus		**Bearded seal**		
	M	F	M	F	U
FA composition					
2009	4	0	3	5	2
2010	8	11	5	15	0
$\delta^{13}C_{FA}$					
2009	4	0	3	5	2
2010	8	28	5	15	0
2011	1	8	0	0	0

Table 3.2 Relative proportions (% total) of benthic fatty acid markers in walruses and bearded seals, including individual non-methylene interrupted fatty acids, their sum, and a composite bacterial fatty acid marker. Letters a-b indicate significant differences between mean values for each species (Two-factor nested ANOVA, $P < 0.01$). Values are means (1SD) with sexes pooled. *22:2NMID varied significantly between male and female walruses (NMID= non-methylene interrupted dienoic fatty acids). The composite bacterial marker consists of the sum of the fatty acids *ai*-15:0, *i*-15:0, *ai*-17:0, *i*-17:0.)

	Pacific walrus	**Bearded seal**
20:2Δ5,11	0.29 (0.08)[a]	0.02 (0.02)[b]
20:2Δ5,13	0.16 (0.06)[a]	0.08 (0.02)[b]
20:3Δ5,11,14	0.06 (0.02)[a]	0.03 (0.01)[b]
22:2NMID*	0.08 (0.04)[a]	0.06 (0.02)[b]
22:2Δ7,13	0.20 (0.08)[a]	0.11 (0.06)[b]
22:2Δ7,15	0.08 (0.03)[a]	0.49 (0.11)[b]
Sum (NMI)	0.87 (0.23)[a]	0.78 (0.13)[a]
Sum (Bacterial)	0.97 (0.21)[a]	0.80 (0.09)[b]

Table 3.3 Estimates of the proportional contributions (%) of sympagic, pelagic and benthic particulate organic matter to consumer diets. Values are based on stable isotope mixing models run *without* and *with* concentration dependencies (Appendix 3.3) (means (95 % credibility intervals)). Particulate organic matter (POM) includes sympagic (ice) (i-POM), pelagic (p-POM), and benthic (b-POM) sources. Male (M) and female (F) walruses (*O. r. divergens*) were analyzed separately and combined, whereas sexes were combined for bearded seals (*E. barbatus*). We posit that model estimates highlighted in bold are most reliable estimates based on sample size and incorporation of concentration dependencies.

i-POM	**Pacific walrus** (M)	**Pacific walrus** (F)	**Pacific walrus (Pooled)**	**Bearded seal (Pooled)**
without				
16:1n-7, 20:5n-3, 22:6n-3	14 (0-28)	19 (0-36)	14 (0-26)	44 (36-52)
16:1n-7, 20:5n-3	26 (4-45)	19 (0-41)	23 (2-40)	44 (33-55)
16:1n-7, 22:6n-3	15 (0-29)	23 (2-42)	15 (2-28)	47 (38-56)
20:5n-3, 22:6n-3	10 (0-23)	17 (4-29)	13 (2-23)	40 (31-49)
with				
16:1n-7, 20:5n-3, 22:6n-3	8 (0-25)	15 (0-37)	14 (0-26)	31 (16-48)
16:1n-7, 20:5n-3	12 (0-36)	13 (0-35)	23 (2-40)	20 (1-64)
16:1n-7, 22:6n-3	15 (0-34)	26 (1-47)	15 (2-28)	18 (8-31)
20:5n-3, 22:6n-3	**7 (0-20)**	**8 (0-19)**	**13 (2-23)**	**27 (15-42)**
p-POM				
without				
16:1n-7, 20:5n-3, 22:6n-3	37 (6-66)	49 (25-75)	45 (23-70)	5 (0-12)
16:1n-7, 20:5n-3	38 (8-69)	52 (26-80)	53 (27-81)	4 (0-10)
16:1n-7, 22:6n-3	21 (0-42)	37 (10-62)	27 (7-46)	5 (0-13)
20:5n-3, 22:6n-3	67 (41-91)	60 (44-74)	67 (54-80)	15 (3-27)
with				
16:1n-7, 20:5n-3, 22:6n-3	36 (10-60)	46 (20-73)	42 (21-61)	8 (0-17)
16:1n-7, 20:5n-3	42 (14-68)	50 (25-78)	49 (30-68)	5 (0-14)
16:1n-7, 22:6n-3	22 (2-44)	35 (7-62)	23 (4-43)	10 (0-24)
20:5n-3, 22:6n-3	**58 (31-90)**	**47 (27-69)**	**51 (32-73)**	**10 (1-22)**
b-POM				
without				
16:1n-7, 20:5n-3, 22:6n-3	49 (15-83)	32 (3-57)	41 (13-67)	51 (39-62)
16:1n-7, 20:5n-3	35 (0-69)	28 (0-54)	23 (0-50)	53 (39-66)
16:1n-7, 22:6n-3	64 (35-93)	40 (9-70)	58 (33-83)	48 (35-60)
20:5n-3, 22:6n-3	23 (0-48)	24 (6-41)	20 (5-36)	45 (30-60)
with				
16:1n-7, 20:5n-3, 22:6n-3	56 (28-86)	39 (6-68)	54 (32-76)	61 (37-81)
16:1n-7, 20:5n-3	46 (17-76)	37 (4-63)	47 (26-67)	75 (28-97)
16:1n-7, 22:6n-3	63 (28-94)	39 (4-74)	63 (30-92)	72 (48-90)
20:5n-3, 22:6n-3	**35 (1-62)**	**45 (17-71)**	**44 (17-67)**	**62 (38-83)**

3.10 Figures

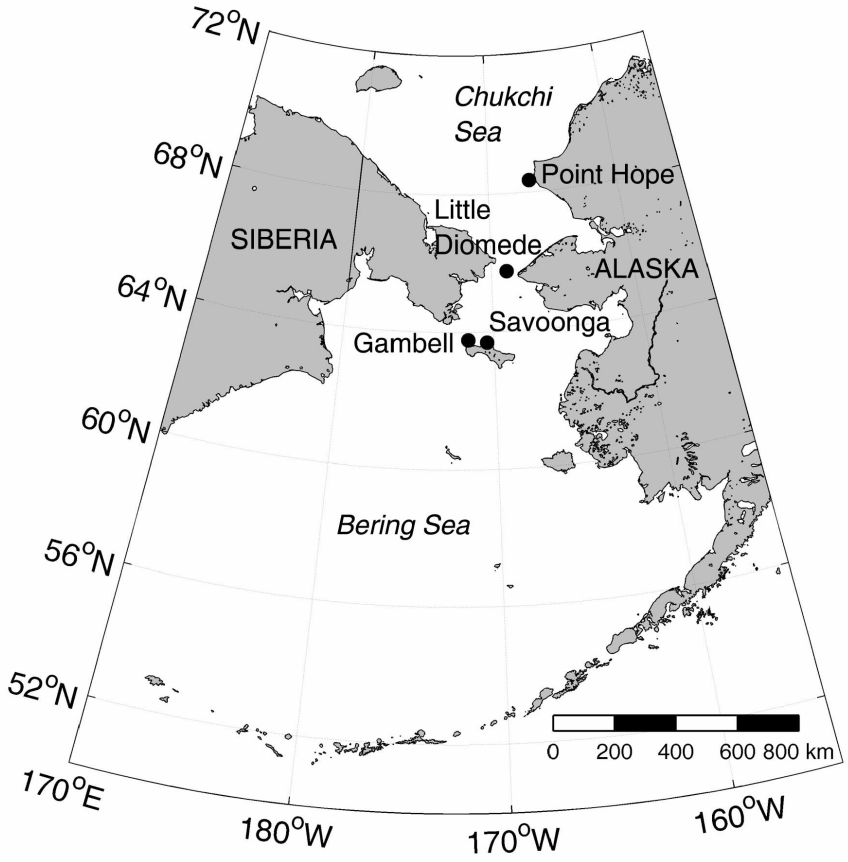

Figure 3.1 Locations of Alaskan communities where Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*) were harvested in 2009-2011. Information on specimens and analyses is provided in Table 3.1.

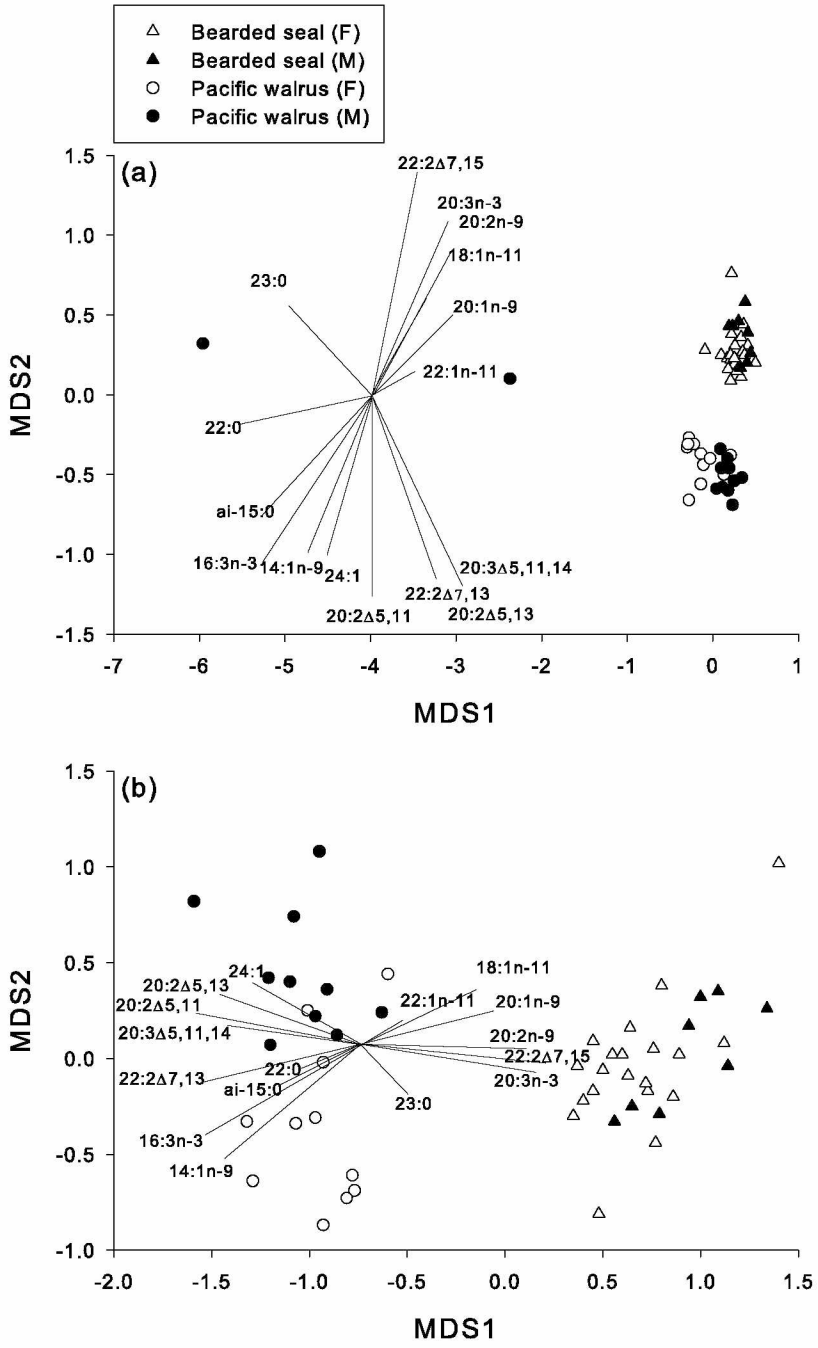

Figure 3.2 Non-metric multidimensional scaling plots of Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*) with (a) walrus outlier specimens included and (b) excluded). Distances are based on Bray-Curis similarity matrices using 77 fatty acids occurring in relative proportions > 0.1 %. Fatty acid vectors displayed are those that contributed most to differences between species and accounted for 45 % of the dissimilarity (SIMPER). We set the level of dissimilarity to 45 % to include all but one non-methylene interrupted fatty acid. Vector length and direction correspond to the strength of correlation with nMDS axes. 2D stress = 0.1 when outliers (n = 2) were included (a). 2D stress = 0.07 when outliers were excluded (b). (M = males, F = females).

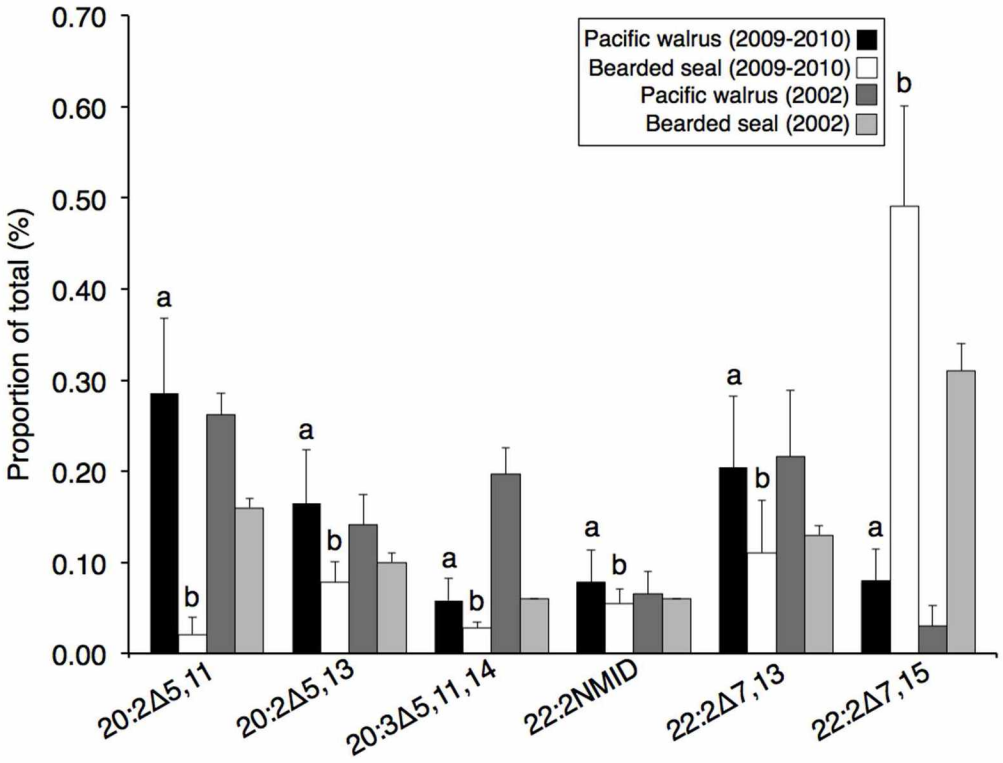

Figure 3.3 Relative proportions (% total) of non-methylene interrupted fatty acids in Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*). Values are means ± 1SD, with sexes pooled from 2009-2010 (this study) and from 2002 (Budge et al. 2007; Cooper et al. 2009). Letters a-b indicate significant differences between species (Two-factor nested ANOVA, $P < 0.01$).

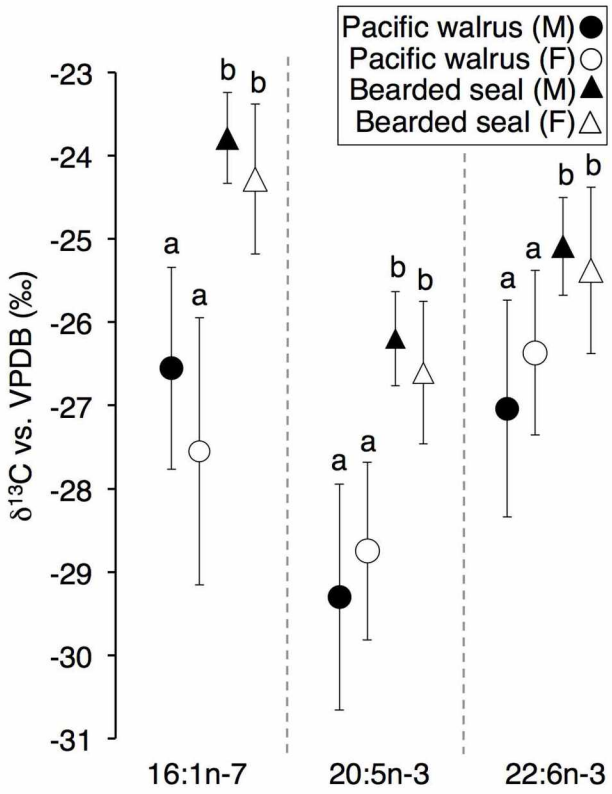

Figure 3.4 $\delta^{13}C$ values (‰) for algal marker fatty acids from Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*). Algal marker fatty acids are 16:1n-7, 20:5n-3, and 22:6n-3 (mean ± 1SD, sexes pooled). Letters a-b indicate significant differences between species and sexes (Two-factor nested ANOVA, $P < 0.01$) (M = males, F = females).

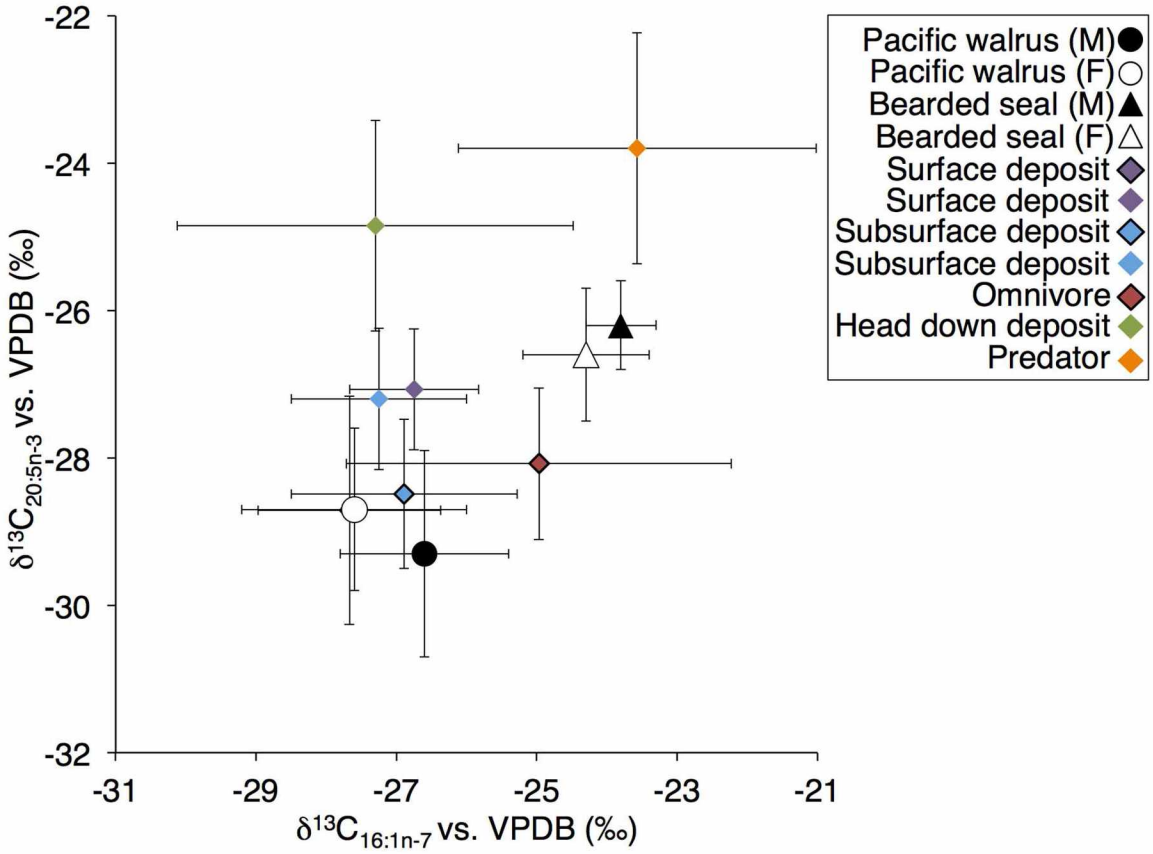

Figure 3.5 $\delta^{13}C$ values (‰) of algal marker fatty acids (16:1n-7 and 20:5n-3) from Pacific walruses (*O. r. divergens*), bearded seals (*E. barbatus*), and benthic invertebrate prey from distinct feeding groups. Prey taxa were collected from the Chukchi Sea (symbols outlined in black, this study) and Bering Sea (Oxtoby et al. in revision). Surface deposit = suspension/surface deposit-feeding bivalves *Liocyma fluctuosa*, *Serripes groenlandica*, *Astarte* spp., *Macoma* spp., and *Ennucula tenuis* (this study), Surface deposit = *Macoma calcarea* and *Ennucula tenuis* (Oxtoby et al. in revision), Subsurface deposit = subsurface deposit-feeding bivalve *Nuculana radiata*, Omnivore = omnivorous crab *Chionoecetes opilio,* Head down deposit = head down deposit-feeding polychaete *Leitoscoloplos pugettensis*, Predator = predatory polychaete *Nephtys* spp.

Appendix 3.1 Relative proportions (% total) of fatty acids from Pacific walruses (*O. r. divergens*) and bearded seals (*E. barbatus*). Values are means (1SD) for fatty acids that were present in > 0.1 % total (M = males, F = females).

	Pacific walrus (M)	**Pacific walrus** (F)	**Bearded seal** (M)	**Bearded seal** (F)
n	12	11	8	20
14:0	3.9 (1.2)	3.1 (0.3)	2.5 (0.4)	2.5 (0.4)
14:1n-9	0.2 (0.0)	0.3 (0.1)	0.1 (0.0)	0.1 (0.1)
14:1n-5	0.4 (0.1)	0.3 (0.1)	0.8 (0.2)	0.7 (0.2)
i-15:0	0.1 (0.2)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
ai-15:0	0.1 (0.1)	0.2 (0.2)	0.1 (0.0)	0.1 (0.0)
15:0	0.5 (0.0)	0.5 (0.1)	0.3 (0.0)	0.4 (0.1)
16:0	12.1 (2.9)	10.9 (0.8)	7.2 (0.9)	7.6 (1.4)
16:1n-11	0.3 (0.1)	0.3 (0.0)	0.3 (0.1)	0.3 (0.1)
16:1n-9	0.4 (0.1)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)
16:1n-7	20.7 (12.6)	21.5 (3.7)	20.7 (2.2)	20.8 (3.4)
16:1n-5	0.3 (0.1)	0.2 (0.0)	0.3 (0.0)	0.3 (0.0)
i-17:0	0.4 (0.1)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)
ai-17:0	0.3 (0.1)	0.3 (0.0)	0.3 (0.0)	0.3 (0.0)
16:2n-4	0.5 (0.1)	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)
17:0	0.3 (0.1)	0.4 (0.1)	0.2 (0.0)	0.3 (0.1)
16:3n-4	0.2 (0.0)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)
17:1	0.4 (0.1)	0.5 (0.1)	0.6 (0.1)	0.6 (0.1)
16:4n-1	0.3 (0.1)	0.3 (0.1)	0.3 (0.2)	0.3 (0.2)
18:0	2.3 (0.8)	2.3 (0.3)	1.3 (0.3)	1.6 (0.4)
18:1n-13	0.4 (0.1)	0.6 (0.1)	0.5 (0.1)	0.5 (0.1)
18:1n-11	0.3 (0.2)	0.2 (0.1)	0.6 (0.2)	0.5 (0.2)
18:1n-9	13.6 (5.3)	10.3 (2.9)	16.5 (2.1)	15.4 (2.8)
18:1n-7	8.5 (2.7)	10.7 (2.1)	9.7 (1.5)	9.6 (1.3)
18:1n-5	0.4 (0.1)	0.4 (0.1)	0.6 (0.1)	0.5 (0.1)
18:2n-6	0.6 (0.1)	0.7 (0.2)	0.8 (0.1)	0.9 (0.1)
18:2n-4	0.3 (0.1)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)
18:3n-4	0.3 (0.1)	0.4 (0.0)	0.3 (0.0)	0.3 (0.0)
18:3n-3	0.1 (0.0)	0.1 (0.0)	0.3 (0.0)	0.3 (0.1)
18:4n-3	0.6 (0.2)	0.7 (0.2)	0.7 (0.2)	0.7 (0.2)
18:4n-1	0.3 (0.1)	0.4 (0.0)	0.3 (0.0)	0.3 (0.1)
20:1n-11	2.1 (0.9)	2.0 (0.3)	1.6 (0.2)	1.7 (0.3)
20:1n-9	1.4 (0.6)	0.8 (0.2)	2.2 (0.6)	2.1 (0.8)
20:1n-7	4.4 (1.6)	4.7 (0.7)	2.3 (0.6)	3.0 (0.7)
20:2Δ5,11	0.3 (0.1)	0.3 (0.0)	0.0 (0.0)	0.0 (0.0)
20:2Δ5,13	0.2 (0.1)	0.2 (0.0)	0.1 (0.0)	0.1 (0.0)
20:2n-6	0.3 (0.1)	0.3 (0.0)	0.3 (0.0)	0.4 (0.0)
20:3Δ5,11,14	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)
20:3n-6	0.2 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.0)
20:4n-6	0.5 (0.1)	0.8 (0.2)	1.1 (0.2)	1.0 (0.1)
20:4n-3	0.5 (0.2)	0.6 (0.1)	0.4 (0.1)	0.5 (0.1)
20:5n-3	6.1 (1.2)	9.3 (1.4)	8.6 (1.7)	7.5 (1.5)
22:1n-11	0.3 (0.3)	0.1 (0.1)	0.3 (0.2)	0.3 (0.2)
22:1n-9	0.2 (0.1)	0.3 (0.1)	0.2 (0.0)	0.2 (0.1)
22:2NMID	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.1 (0.0)
22:2Δ7,13	0.2 (0.1)	0.2 (0.1)	0.1 (0.0)	0.1 (0.1)
22:2Δ7,15	0.1 (0.0)	0.1 (0.0)	0.5 (0.1)	0.5 (0.1)
21:5n-3	0.8 (0.3)	0.7 (0.2)	0.5 (0.1)	0.6 (0.1)
22:4n-6	0.2 (0.1)	0.2 (0.0)	0.3 (0.0)	0.3 (0.1)
22:5n-6	0.2 (0.1)	0.3 (0.1)	0.2 (0.0)	0.3 (0.1)
22:5n-3	6.4 (2.5)	5.7 (0.9)	4.7 (0.6)	5.3 (0.6)
22:6n-3	4.7 (1.9)	3.8 (1.1)	7.7 (1.4)	7.7 (1.7)

Appendix 3.2 Relative proportions (% total) and $\delta^{13}C$ values (‰) of algal marker fatty acids from benthic invertebrate prey. Algal marker fatty acids are 16:1n-7 and 20:5n-3 (mean (1SD), samples pooled by feeding group). Benthic prey consist of an omnivore (*Chionoecetes opilio*), a subsurface deposit-feeder (*Nuculana radiata*), and suspension/surface deposit-feeders (*Liocyma fluctuosa*, *Serripes groenlandica*, *Astarte* spp., *Macoma* spp., and *Ennucula tenuis)*.

Source	**16:1n-7**		**20:5n-3**	
	Mean (SD) %	Mean (SD) ‰	Mean (SD) %	Mean (SD) ‰
Omnivore (n = 36)	10.9 (4.7)	-25.0 (2.7)	14.9 (4.8)	-28.1 (1.0)
Subsurface deposit-feeder (n = 42)	26.0 (7.5)	-26.9 (1.6)	15.8 (4.0)	-28.5 (1.0)
Suspension/surface deposit-feeder (n = 82)	19.0 (11.0)	-27.7 (1.3)	15.5 (7.1)	-28.7 (1.5)

Appendix 3.3 Relative proportions (% total) and $\delta^{13}C$ values (‰) of algal marker fatty acids from sympagic, pelagic, and benthic particulate organic matter sources for stable isotope mixing models in walruses and bearded seals. Algal marker fatty acids are 16:1n-7, 20:5n-3, and 22:6n-3 (mean ± 1SD, samples pooled by algal source). Particulate organic matter sources include sympagic (ice) (i-POM), pelagic (p-POM), and benthic (b-POM) fatty acids.

Source	**16:1n-7**		**20:5n-3**		**22:6n-3**	
	Mean (SD) %	Mean (SD) ‰	Mean (SD) %	Mean (SD) ‰	Mean (SD) %	Mean (SD) ‰
i-POM (n = 21)	15.7 (6.7)	-24.9 (4.6)	20.1 (6.8)	-26.3 (2.8)	2.3 (0.8)	-23.3 (2.8)
p-POM (n = 55)	16.2 (12.9)	-29.7 (1.7)	11.7 (7.0)	-29.3 (1.6)	4.9 (3.2)	-27.3 (2.5)
b-POM (n = 79)	27.8 (16.1)	-26.7 (1.0)	3.5 (2.4)	-26.9 (1.7)	1.0 (0.8)	-27.2 (2.1)

Appendix 3.4 Permission from co-author Tanja Schollmeier to include this manuscript in the dissertation.

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Laura Oxtoby <laura.oxtoby@gmail.com>

Permission to use manuscript in my dissertation

Tanja Schollmeier <tanja.schollmeier@gmail.com> Mon, Feb 8, 2016 at 11:21 AM
To: Laura Oxtoby <laura.oxtoby@gmail.com>
Cc: Tanja S <tschollmeier@alaska.edu>

Hi Laura,

no problem, you of course have permission! Good luck.

Cheers,
Tanja

On Mon, Feb 8, 2016 at 11:02 AM, Laura Oxtoby <laura.oxtoby@gmail.com> wrote:

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>
> You are a co-author on a manuscript that I would like to include in my dissertation to fulfill the requirements of a PhD in Marine Biology from the University of Alaska Fairbanks. It is titled, "Resource partitioning between Pacific walruses and bearded seals during 2009-2011 in Alaska".
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> Laura
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> --
> Laura Oxtoby
> PhD candidate
> School of Fisheries and Ocean Sciences
> University of Alaska Fairbanks
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Appendix 3.5 Permission from co-author Shiway Wang to include this manuscript in the dissertation.

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Laura Oxtoby <laura.oxtoby@gmail.com>

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Shiway Wang <shiway@gmail.com> Mon, Feb 8, 2016 at 11:14 AM
To: Laura Oxtoby <laura.oxtoby@gmail.com>

Permission granted!

On Mon, Feb 8, 2016 at 11:02 AM, Laura Oxtoby <laura.oxtoby@gmail.com> wrote:

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> You are a co-author on a manuscript that I would like to include in my dissertation to fulfill the requirements of a PhD in Marine Biology from the University of Alaska Fairbanks. It is titled, "Resource partitioning between Pacific walruses and bearded seals during 2009-2011 in Alaska".
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>
> Thanks,
> Laura
>
> --
> Laura Oxtoby
> PhD candidate
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> University of Alaska Fairbanks
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General Conclusions

Identifying carbon sources and assigning their roles in food web dynamics is of fundamental importance to forecast community-level consequences and species-specific responses to environmental change that may affect these carbon sources. This dissertation extended the use of stable isotope analysis through modeling and compound-specific applications to elucidate organic matter (OM) sources, trophic connectivity, and resource partitioning in benthic food webs in the Alaska Arctic and sub-Arctic.

Chapter 1 combined a rare set of field measurements of bottom water dissolved inorganic carbon (DIC) with previously published physiological relationships from algal culture studies (Laws et al. 1995; Popp et al. 1998) to model the bounds of $\delta^{13}C$ values of microphytobenthos. Although microphytobenthos can be collected opportunistically, it has not been successfully isolated for stable isotope analysis from high-latitude environments to date. The $\delta^{13}C$ values presented here were based on the best available data to constrain the isotopic composition of microphytobenthos from high-latitude ecosystems. This novel modeling approach mitigated the potential for contamination from meiofauna, bacteria, and benthic detritus on the $\delta^{13}C$ values of benthic algal production from actual field measurements. Modeled values for microphytobenthos were isotopically similar to pelagic particulate organic matter (POM) from the circumpolar Arctic (e.g., Iken et al. 2010) but were distinct from riverine POM and ice algal POM from Alaska waters (e.g., Dunton et al. 2012; Søreide et al. 2013). If modeled estimates accurately reflect the stable isotopic composition of microphytobenthos, previous benthic food web models in the Alaska Arctic (e.g., Iken et al. 2005; Lovvorn et al. 2005; McTigue and Dunton 2014) may have underestimated algal contributions originating in the benthic environment.

Chapter 2 used a multi-proxy approach consisting of fatty acid (FA) profiles, FA markers, and compound-specific stable isotope analysis of algal marker FAs to demonstrate that FA sources of key benthic invertebrate taxa differed by feeding strategy. Focal species included two suspension/surface deposit-feeding bivalves (*Macoma calcarea*, *Ennucula tenuis*), a subsurface deposit-feeding bivalve (*Nuculana radiata*), a head-down deposit-feeding polychaete, and a predatory polychaete (*Nephtys* spp.). I accounted for benthic FA sources by including FAs from surface sediment POM and provided evidence for the influence of benthic processes (i.e., microbial degradation of algal detritus) on the chemical composition of FAs available to deposit-

feeders. This research revealed that benthic consumers with unique feeding ecologies relied on distinct FA sources in the Bering Sea in 2009-2010, an indication of low interspecific competition.

Chapter 3 applied the multi-proxy, analytical compound-specific stable isotope analysis approach from Chapter 2 to investigate benthic resource partitioning between Pacific walruses and bearded seals. Based on FA profiles and markers, and $\delta^{13}C$ values of algal FAs, I established that these bottom-feeding pinnipeds relied on different benthic prey taxa. Comparisons of non-methylene interrupted FAs (benthic markers) (Budge et al. 2007) measured in 2002 (Cooper et al. 2009), were strikingly similar to those I measured for 2009-2010, despite the fact that 2002 was a warm year in the Bering Sea and 2009-2010 were cold years in the Bering Sea (Stabeno et al. 2012). In the Bering Sea, warm years are characterized by higher water temperatures and low ice extent, whereas cold years are characterized by lower water temperatures and higher ice extent (Stabeno et al. 2012). These environmental conditions influence Arctic sea ice phenology and primary production, which may affect benthic prey communities that support populations of Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). This dataset provides a valuable baseline from which to measure future, potential changes in resource competition between these apex predators.

There are several inherent challenges to studying trophic connectivity in the Arctic and sub-Arctic benthic environment that necessitate consideration in future research efforts. First, there are myriad sources of algal OM available to benthic consumers, the extent of one of which, under-ice phytoplankton, was only recently discovered (Arrigo et al. 2012). Furthermore, not all of these sources have been analyzed for their chemical composition (i.e., stable isotope and FA). Second, OM is subject to processes of degradation that can alter its chemical composition (e.g., Dai et al. 2005; Leu et al. 2010). Finally, we have a poor understanding of the influence of organismal physiology on the assimilation of algal OM.

The stable carbon isotope composition of several algal sources in the Alaska Arctic and sub-Arctic marine environment is currently unknown. These include, but are not limited to, microphytobenthos, under-ice phytoplankton, and OM from subsurface sediments. To improve our understanding of benthic food web dynamics, we need to collect, analyze, and include these sources of OM in future stable isotope mixing models. Revised estimates of the relative

importance of various sympagic, pelagic, and benthic algal sources to total annual primary production in the Arctic and sub-Arctic marine environment would also greatly inform estimates of proportional contributions of algal sources to consumers. Few estimates of primary production exist for the Arctic (Legendre et al. 1992; Gosselin et al. 1997) and those that do are outdated as they do not reflect newly discovered sources or changes in the sea ice environment over the last decade (e.g., Brown and Arrigo 2012; Ray et al. 2015).

Algal biomass is subject to processes of degradation in the water column and in the sediment. In Chapter 2, I posited that FAs of algal origin may have a unique stable isotope composition due to fractionation associated with microbial oxidation of algal FAs (Sun et al. 2004). Previous research from an estuarine ecosystem revealed differences in isotopic fractionation of individual FAs under oxic and anoxic conditions (Dai et al. 2005, 2009). However, controlled degradation experiments that examine FA isotopic fractionation in algae and sediments from our study regions are necessary to test this hypothesis. Quantification of microbial abundance and biomass in shelf sediments in the Arctic and sub-Arctic would help to elucidate the role of microbes in carbon cycling and help to improve interpretations of consumer and sediment FAs and their $\delta^{13}C$ values.

Stable isotope food web models (including the compound-specific Bayesian multiple source models employed in Chapters 2 and 3) rely on several key assumptions regarding the assimilation of OM by a consumer. The first assumption is that there is neglible or predictable isotopic fractionation from prey or carbon source to consumer (a trophic enrichment factor-TEF). TEFs of FAs have not been established for any of the species included in this dissertation or in any closely related species. Recent controlled feeding studies have investigated trophic fractionation factors for FAs, including the algal marker FAs I examined (20:5n-3 and 22:6n-3), in Steller's eiders (*Polysticta stelleri*) and spectacled eiders (*Somateria fischeri*) (Budge et al. 2011), mud snails (*Bellamya chinensis*) and zebrafish (*Danio rerio*) (Fujibayashi et al. 2016), and water fleas (*Daphnia galeata*) (Gladyshev et al. 2016). Findings from these experiments indicated that TEFs for FAs are highly variable and depend on diet, the FAs in question, and the consumer. One mechanism proposed for isotopic fractionation between diet and consumer is synthesis of long chain polyunsaturated FAs, such as 20:5n-3 and 22:6n-3, from precursor FAs by consumers. While I assume that the abundant dietary supply of algal FAs is a probable

disincentive to synthesis from precursors, I cannot exclude the possibility that synthesis occurs. Furthermore, analyses of benthic invertebrate genomes indicated that a broad range of taxa have the genetic instructions to code for necessary elongase and desaturase enzymes (Monroig et al. 2013 and references therein). However, whether those genes are expressed can depend on dietary supply (reviewed in Monroig et al. 2013). Lastly, another uncertainty is the rate of FA turnover in consumers. The length of time reflected by FA $\delta^{13}C$ values from an organism's lipid reserve has not been established for any of the species investigated in this dissertation. In the absence of controlled feeding studies or tracer experiments, observations of foraging or knowledge of established life history patterns can provide complementary lines of evidence with which to constrain our understanding of diet. However, further research is necessary in these areas to refine the use of compound-specific stable isotope analyses for future food web modeling efforts.

In conclusion, stable isotope analysis was used to infer trophic relationships and inform a broader picture of benthic ecosystem structure and function in the Arctic and sub-Arctic benthic environment surrounding Alaska. Stable isotope analysis is a powerful analytical tool in remote regions where food web observations and sample collection can be challenging and, at times, opportunistic. Compound-specific analysis of algal FA markers can link algal production from the ice environment, open ocean, and sediment, to food web consumers, a research objective that will only increase in its importance as the ice environment continues to change.

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